Intrathecal Catheterization and Drug Delivery in Guinea Pigs

A Small-animal Model for Morphine-evoked Granuloma Formation

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ABSTRACT

Background: Intrathecal infusion of opioids in dogs, sheep, and humans produces local space-occupying masses. To develop a small-animal model, the authors examined effects of intrathecal catheterization and morphine infusion in guinea pigs.

Methods: Under isoflurane, polyethylene or polyurethane catheters were advanced from the cisterna magna to the lumbar enlargement. Drugs were delivered as a bolus through the externalized catheter or continuously by subcutaneous minipumps. Hind paw withdrawal to a thermal stimulus was assessed. Spinal histopathology was systematically assessed in a blinded fashion. To assist in determining catheter placement, *ex vivo* images were obtained using magnetic resonance imaging in several animals. Canine spinal tissue from previous intrathecal morphine studies was analyzed in parallel.

Results: (1) Polyethylene (n = 30) and polyurethane (n = 25) catheters were implanted in the lumbar intrathecal space. (2) Bolus intrathecal morphine produced a dose-dependent (20 to 40 μg/10 μl) increase in thermal escape latencies. (3) Absent infusion, a catheter-associated distortion of the spinal cord and a fibrotic investment were noted along the catheter tract (polyethylene > polyurethane). (4) Intrathecal morphine infusion (25 mg/ml/0.5 μl/h for 14 days) resulted in intrathecal masses (fibroblasts, interspersed collagen, lymphocytes, and macrophages) arising from meninges proximal to the catheter tip in both polyethylene- and polyurethane-catheterized animals. This closely resembles mass histopathology from intrathecal morphine canine studies.

Conclusions: Continuous intrathecal infusion of morphine leads to pericatheter masses that morphologically resemble those observed in dogs and humans. This small-animal model may be useful for studying spinal drug toxicology in general and the biology of intrathecal granuloma formation in particular. **(Anesthesiology 2016; 125:378-94)**

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SINCE 1991, numerous clinical case series describe patients receiving intrathecal morphine infusion who present with neurologic signs secondary to a local compressive lesion. Retrospective data indicate an overall incidence of 0.1%,[1] although estimates in limited populations have been as high as 43%.[2]

Studies in chronic intrathecally catheterized dogs[3–5] and sheep[6] demonstrated that intrathecal morphine, infused over 14 to 28 days, reliably produced local masses proximal to the infusion site. An important observation that we made was that the cellular mass arose from the dura/arachnoid layer and *not* from the parenchyma.[4] Serial magnetic resonance images showed that termination of morphine resulted in a progressive reduction in mass size.[7] Several variables characterize the formation of the meningeal mass, including higher drug concentration (*vs.* total dose),[8] lack of an effect mediated by an opioid receptor,[7,9] and an intermediary role of meningeal mast cell degranulation.[9]

What We Already Know about This Topic

- Intrathecal infusion of morphine is associated with concentration and dose-dependent risks of granuloma formation and neurologic symptoms in large mammals and humans
- Assessment of the mechanisms by which intrathecal morphine induces granulomas would be facilitated by a small-animal model

What This Article Tells Us That Is New

- In guinea pigs, intrathecal morphine infusion produced granuloma formation with similar characteristics as observed in humans, suggesting the utility of the study of mechanisms of these adverse events in these animals

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Preclinical work has provided insight into the potential mechanisms of granuloma formation. One limitation has been that the preclinical work has employed large-animal models (*e.g.*, dogs and sheep). While these models have great utility for defining spinal drug distribution/pharmacokinetics and provide an important tool for defining preclinical safety before human trials,[10] these models are costly and difficult to implement for routine preclinical screening of novel spinal agents. We considered a smaller species and chose to focus on the guinea pig (*Cavea porcellus*). This animal, said by some to be a nonrodent species,[11,12] possesses great similarity to humans with regard to the pathophysiology of a variety of diseases and shares significant similarity to humans with regard to innate immunology and complement system (see Ref. [13]).

We sought to determine if, like dog, sheep, and human, guinea pigs, in response to the continuous intrathecal infusion of morphine, would develop a pericatheter granuloma. Meningeal mast cells have been identified in the guinea pig.[14,15] Not unexpectedly, μ-opioid binding has been identified in the guinea pig spinal dorsal horn.[16,17] Previous work has shown that the guinea pig shows robust thermal escape[18] and an analgesic response to opiates.[19] However, only two studies describe the spinal delivery of agents in guinea pigs, one examining epidural catheterization and effects of local anesthetics[20,21] and one employing bolus percutaneous delivery.[22] We undertook to characterize spinal reactions to chronic intrathecal catheterization as a function of catheter material and the analgesic effects of intrathecal morphine with systematic attention to the pathology associated with the intrathecal infusion of morphine. Moreover, we compare the morphology of the guinea pig mass to that observed in the canine model. The current results provide confirmation that the guinea pig is an appropriate model to study morphine-induced analgesia and granuloma formation.

Materials and Methods

These studies were carried out according to a protocol approved by the Institutional Animal Care and Use Committee at the University of California, San Diego, California.

Animals

Adult male Hartley guinea pigs (275 to 300 g) were purchased from Charles River Labs, USA. Upon receipt, animals were pair-housed in standard cages and maintained on a 12:12-hr light/dark cycle. After a minimum of 2 days of acclimation, animals were entered into the study and prepared with chronic lumbar intrathecal catheters. For bolus delivery, the catheters were externalized. For continuous infusion, the catheter was passed subcutaneously and connected to subcutaneously implanted Alzet osmotic minipumps (Model 2002, DURECT Corporation, USA). All of the procedures and testing were conducted during the light cycle of the day. Food and water were freely available.

Behavioral Testing

On day 0, animals prepared with catheters were randomly selected for study. Before initiation of drug delivery, baseline behavioral and testing data were taken. At selected times after infusion of the test or control article, these data were again collected. All assessments were made with observer blinded to drug treatment. The animals were euthanized upon completion of the final test period.

Acute Thermal Escape

A hind paw thermal stimulator system was employed, first described by Hargreaves,[23] constructed in the engineering lab in the Department of Anesthesiology at the University of California, San Diego.[24] This system allowed the direction of a focused light beam on the plantar surface of the paw, through a glass plate upon which the guinea pig stood. Surface temperature was maintained at 28°C. The guinea pigs were placed on the thermal escape box and allowed to acclimate for 30 min before testing. A brisk withdrawal of the paw was taken as the response. Lack of a response within 20 s was cause to terminate the test and assign the score of "20 s." A latency measurement was taken for the right and left hind paws and averaged. Measurements were then made at various time points after drug administration. Each time point signifies the time at which the hind paws were tested. Unless otherwise stated, animals were assessed for thermal escape latencies at baseline and again at 30, 60, and 120 min after bolus injection or 1, 3, 7, 10, 14, 15, and 16 days after initiation of infusion.

Behavioral Assessment

General behavioral assessments were made during each period of observation. Observations included corneal reflex, pinna reflex, gross motor assessment, and scratching. All assessments were noted as "present," "absent," "within normal limits," or "abnormal."

Catheter Construction

Intrathecal polyethylene catheters were constructed from two pieces of medical-grade polyethylene tubing. Catheters used for continuous infusion were fitted with an additional length of polyethylene or polyurethane tubing to facilitate connection to the Alzet pumps. The implanted portion (0.20-mm ID × 0.36-mm OD; Scientific Commodities, Inc., USA, #BB31695-polyethylene/08) was heat fused to the externalized portion (0.28-mm ID × 0.64-mm OD; Scientific Commodities, Inc., #BB31695-polyethylene/1). For adaptation to Alzet pumps, the externalized portion of the catheter was heat fused to an additional length of polyethylene tubing (0.76-mm ID × 1.22-mm OD; Scientific Commodities, Inc., #BB31695-polyethylene/4). Catheters were packaged and sterilized by ethylene oxide before use. The polyurethane catheter was obtained commercially. The intrathecal component was constructed from polyurethane (0.13-mm ID × 0.27-mm OD), and the external component was polyurethane (0.30-mm ID × 0.64-mm OD). The two components

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of the catheter were joined by a bonding agent. A stainless steel Teflon-coated stylet was fed into the catheter lumen. Catheters intended for attachment to the Alzet pump were bonded to an additional short piece of polyurethane tubing (0.64-mm ID × 1.18-mm OD). Each catheter was individually packaged and ethylene oxide sterilized (Product Protocol 2014-06A, ReCathCo, USA). The physical properties of the implanted portions of the polyethylene and polyurethane catheters were assessed at room temperature using the flexure test, which measures behavior of materials subjected to simple beam loading (see Supplemental Digital Content 1, http://links.lww.com/ALN/B289, a table listing the physical properties of polyethylene and polyurethane tubing). The data generated emphasize the much greater flexibility of the polyurethane catheter as compared to similarly sized polyethylene catheter.

Surgical Preparation

For catheter insertion, the animals were sedated with ketamine (20 mg/kg, intraperitoneal) and xylazine (2 mg/kg, intraperitoneal). The back of the neck was shaved and surgically prepped. The animal was mounted in a rodent stereotaxic head holder with ear bars and fitted with a facemask for delivery of oxygen and isoflurane (2%). All animals were monitored during the procedure to ensure adequate anesthetic depth and adjust as required. All animals presented with loss of motor tone and were without response to deep pain. Aseptic precautions were followed (facemask, hair cover, sterile gown, and sterile instrument packs). For implantation, the head was tilted forward to establish exposure of the cisternal membrane. Skin was prepped and draped with a transparent covering (sterilized plastic wrap) to permit continuous visualization of the animal during surgery. The cisternal membrane was exposed by incision and blunt dissection. The catheter was inserted through an incision in the cisternal membrane and passed 9 to 11.5 cm caudally. In preliminary work, we observed in the 300-g male guinea pig that 9-cm catheters were at the spinal level of approximately T12-L1, 10-cm catheters were at the level of T13-L1, 11-cm catheters at L1, and 11.5-cm catheters at L2. For bolus delivery, the catheter was externalized after being tunneled forward on the top of the skull. For continuous infusion, the catheter was tunneled caudally to a site on the upper back where a subcutaneous incision and dissection of a small pocket to hold the osmotic mini pump was made. A polyethylene-60 adapter connected the catheter to the outlet of the pump. The wound was closed using silk (3-0) suture and the animal recovered. Upon terminating the anesthesia, a subcutaneous bolus of lactated Ringer's (1 ml/50 g body weight) was given between the scapulae. An injection of Rimadyl® (5 mg/kg, subcutaneously; Zoetis Inc., USA) was given for postoperative analgesia. The average duration of the implant procedure was 20 to 30 min.

Intrathecal Drugs

Morphine for injection or infusion was prepared from commercially available, preservative-free morphine sulfate for intrathecal use (Morphine Sulfate Inj., USP 25 mg/ml; Hospira, Inc., USA) and diluted using preservative-free sterile saline for injection (0.9% Sodium Chloride Inj., USP; Hospira, Inc.). The NaCl package insert listed the pH between 4.5 and 7.0. To assess opiate antagonism, naloxone HCl (1 mg/kg, intraperitoneal; Sigma-Aldrich, USA) was used.

Histologic Procedures

Guinea pigs were deeply anesthetized with isoflurane anesthetic and given a 1.0 ml intraperitoneal injection of Beuthanasia-D® (Intervet/Schering-Plough Animal Health Corp., USA). They were then transcardially perfused with 1 ml/g of body weight of heparinized saline followed by 1 ml/g of body weight of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). In phase 1, spinal cords were harvested and divided into segments *via* a template; block A (cervical); B (thoracic); C1, C2, and C3 (lumbar); and D1 and D2 (sacral). Spinal sections were placed in tissue cassettes and processed to paraffin. Each phase 2 animal had its spinal column harvested after perfusion fixation and decalcified before paraffin processing. Decalcification was accomplished using Immunocal bone decalcifier (American MasterTech Scientific, Inc., USA) until the column was easily sectioned. This process took 3 to 5 days at room temperature. After decalcification was complete, blocking of the spinal column was accomplished as in the phase I animals, and tissue blocks were processed to paraffin. Tissues were processed and assessed without knowledge of drug treatment.

Paraffin blocks were sectioned at 5 to 10 μm using a Leica 2135 rotary microtome, and sections were slide mounted. Slides were stained with hematoxylin and eosin (H&E) and/or Herovici for young and mature collagen (American MasterTech Scientific, Inc., USA), and/or Astra Blue for mast cells (American MasterTech Scientific, Inc., USA) for further analysis. Two canine lumbar spinal cord blocks from animals displaying a granuloma following intrathecal infusions of morphine (28 days) from a previous study[9] were concurrently examined for H&E, Herovici for collagen, and mast cell staining for comparison with observations made in the guinea pigs. Mast cells in the dura were identified by Astra blue staining following methods provided by the manufacturer (American Master Tech, USA). Brightfield images were taken of each H&E-, Herovici collagen–, and mast cell–stained sections using an Olympus BX51 microscope (Olympus America Inc., USA) and Optronics MagnaFire—SP Digital Camera System.

Immunofluorescence. After perfusion/fixation with 4% paraformaldehyde, spinal cords were isolated, postfixed in 4% paraformaldehyde, cryoprotected in graded sucrose solution, and embedded in Tissue-Tek O.C.T. Compound (Sakura Finetek USA, Inc., USA) on dry ice. Transverse

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sections (10-μm-thick) were blocked for 1 h at 4°C in PBS containing 10% normal goat serum (Vector Laboratories, USA). Sections were incubated for 16 h at 4°C with mouse glial fibrillary acidic protein (GFAP, Cell Signaling, USA, cat #3670, 1:50), rabbit Iba1 (Wako, cat #019-19741, 1:500) antibody, or mouse anti-T cell receptor α/β (TCR, AbD Serotec, USA, cat. #MCA453G, 1:200) antibody, and washed in PBS followed by incubation with the corresponding species-specific secondary Alexa Fluor 488 (green) and Alexa Fluor 594 (red) antibodies (Invitrogen, USA). Slides were mounted in SlowFade Gold Antifade media with 4',6-diamidino-2-phenylindole (DAPI; Life Technologies, USA). Images were acquired using a Digital Module R microscope (Leica Microsystems, USA) and processed using Openlab 4 imaging software (Improvision, USA).

Histologic Assessment Procedures

For pathology assessment, sections judged to be proximal to the catheter tip were selected for grading. Two approaches were employed. Three reviewers independently and without knowledge of treatment first assigned a pathology grade (0 to 4) to each animal based on criteria outlined in Supplemental Digital Content 2A (http://links.lww.com/ALN/B290). Second, each reviewer performed a forced ranking of all representative sections from least to most severe. Scores and ranking were plotted as scatter plots by treatment. If the catheter penetrated the cord, the presence of the parenchymal catheter was noted, but no score was given. For any given animal, the three independent scores for that animal were used to calculate a mean, and this mean rank was used in the subsequent comparisons of treatment and catheter effects.

Postmortem Spinal Column Magnetic Resonance Imaging

Spinal columns, before decalcification, were submitted for magnetic resonance imaging to aid in identifying location of the catheter and mass. After perfusion fixation, 6-cm lengths of the vertebral column (cervical level 1 through sacral level 1) were placed in airtight bags filled with 0.9% NaCl to exclude air bubbles and prevent susceptibility artifacts at the air/tissue interface. Samples were scanned using magnetic resonance imaging (MRI). Images were acquired using a 7 Tesla Bruker (Bruker Biospin Billerica, USA) horizontal-bore small-animal magnet and a 35-mm-diameter imaging volume transmit/receive coil. A T2 TurboRARE sequence was used with the following imaging parameters: echo time/repetition time, 27/11539 ms; flip angle, 180 degrees; field of view, 30 × 30 mm; slice thickness, 0.3 mm; matrix, 300 × 220. The imaging time was 26 min per set of two or three samples.

Study Protocols

Phase 1: Characterization of Dose-dependent Effects of Bolus Intrathecal Morphine. Animals were prepared with polyethylene or polyurethane intrathecal catheters as described previously. After surgery, animals were assessed for general health parameters, behavioral status, motor coordination and function, and muscle-tone parameters. After a 5-day recovery, animals presenting with normal parameters were assessed for acute thermal escape latencies.

Intrathecal Morphine Dose–Response Curves. At each dosing session, each animal received a single 10 μl dose of morphine (20, 30, or 40 μg) or vehicle, which was randomly assigned. This sequence was repeated a total of three times at 3-day intervals to yield a group size of 7 to 9 animals for each dose. In separate studies, animals received naloxone (1 mg/kg, intraperiotoneally) followed 10 min later by the maximum intrathecal morphine dose. After completion of the injection series, each animal was euthanized and given a bolus delivery of dye (10 μl, New Methylene blue "N," 0.5% w/v, Ricca Chemical Company, USA). A spinal dissection was then performed to assess catheter placement and distribution of the injected dye.

Phase 2: Effect of Chronic Infusion of Intrathecal Morphine in the Acute Thermal Escape Model and Spinal Histopathology Associated with Polyethylene and Polyurethane Catheter Placement. Animals were implanted with 11.5-cm polyethylene or polyurethane intrathecal catheters with attached mini osmotic pumps that delivered the test article at 0.5 μl/h for 14 days (Alzet model 2002). Each mini pump was preloaded with morphine (0.25, 2.5, 8, or 25 mg/ml) or vehicle (0.9% NaCl). Thermal latencies were assessed before intrathecal implant and on days 1, 3, 7, 10, 14, 15, and 16 post implant. Additional animals were prepared with polyethylene and polyurethane catheters that were filled with 0.9% NaCl and sealed to permit assessment of the effects of implanted catheters without infusion. Upon completion of final data collection, animals were deeply anesthetized, exsanguinated, and perfusion fixed, and spinal columns/cords were harvested.

Canine Granulomas. For comparison and contrast, paraffin blocks of lumbar spinal cords from previously reported dogs[9] that received intrathecal infusions of morphine (25 mg/ml/20 μl/h for 28 days) were sectioned and stained with H&E, for mast cells and for collagen, using the same protocols that were used for guinea pig sections as described previously.

Statistical Analysis

The data were compiled in Excel (v.14.4.9, Microsoft Corporation, USA), and statistical analyses were performed using Prism (v.6.0, GraphPad Software, Inc., USA). We sought to have a minimum of four animals per group based on previous experience with small-animal spinal drug action on thermal escape and pathology endpoint. In several instances, we have group sizes of 3 as a result of animal or catheter availability.

Bolus Dose–Response Curves for Thermal Escape. Thermal escape latencies in seconds (mean and SDs) over time before and after bolus delivery were plotted. A two-way repeated measures ANOVA was performed with a *post hoc*

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comparison at each time point to baseline for that treatment group using a Bonferroni multiple comparison test. For analysis across doses, the response latencies were normalized by converting them to percentage of the maximum possible effect (Post–Pre)/20 s – Pre) × 100 and the area under the percentage max possible effect curve calculated (AUC). Doses were compared using one-way ANOVA and multiple *t* tests across treatment groups with Tukey correction.

Infusion Effects on Thermal Escape over Time. Two-way repeated measures ANOVA were undertaken on polyethylene groups (morphine *vs.* saline) and polyurethane groups (morphine *vs.* saline). *Post hoc* comparisons were made to baseline using Bonferroni multiple comparisons.

Histopathology. Distribution of individual pathology scores is presented as scatter plots, and data are summarized as mean and SD. As described in the Histologic assessment procedure paragraph under the Methods section, animals receiving a catheter implant without infusion, a catheter with NaCl infusion, or catheter with morphine (25 mg/ml/0.5 µl/h) infusion were rank ordered, and data are summarized as the mean and SD of the ranks. To assess the concordance of the three individual sets of rankings, an intraclass correlation calculator was employed.[25,26] Specific comparisons are made using two-way ANOVA with *post hoc* analysis across treatment groups with Tukey correction. Differences reaching the $P < 0.05$ level of significance were considered to be statistically significant.

Results

In the current study, a total of 30 animals were prepared with polyethylene and 25 with polyurethane lumbar intrathecal catheters. Table 1 summarizes outcome of intended catheter placements by catheter types. As indicated, one animal died intraoperatively. One polyethylene and two polyurethane animals recovered but displayed motor impairment. One animal receiving repeated bolus doses of morphine (polyurethane), one animal implanted with a polyethylene closed-end (no infusion) catheter, and two animals receiving morphine infusion (one polyurethane and one polyethylene) were euthanized at 10, 10, 7, and 10 days post implant for failure to thrive (weight loss and lesions on flank). Four polyurethane catheters disconnected at the tubing junction during the early phase of these studies. This problem was corrected by increasing the volume of the bonding agent.

There was one intraoperative anesthesia-related death (table 1). Animals typically displayed food and fluid consumption by 24 h after surgery and resumed weight gain thereafter.

Phase 1: Bolus Delivery of Morphine Sulfate

Catheter Placement. Twelve animals were entered into the study to receive an intrathecal implant and survived surgery without motor impairment. One animal removed its catheter. One animal's catheter became obstructed 13 days post operation. One animal received its implant successfully and recovered to display morbidity 10 days later that resulted in neurologic deficits, flaccidity, weakness, head tilt, and significant loss of body weight. It underwent euthanasia. All animals were submitted for pathology.

Thermal Escape. The mean (± SD) baseline thermal escape in seconds was 11.8 ± 1.6 for naive animals, 10.3 ± 1.2 for polyurethane animals, and 11.5 ± 0.9 for the polyethylene animals. These differences were not statistically different (one-way ANOVA with *post hoc* Tukey multiple comparison tests, comparing to all treatment groups). Subsequent work revealed no differences between the effects of vehicle and those of morphine delivered by either polyethylene or polyurethane catheters, and these data were merged in subsequent analyses. A bolus intrathecal injection of 0.9% NaCl was without effect on acute thermal latencies (fig. 1A). A bolus intrathecal injection of morphine sulfate (20, 30, or 40 µg) resulted in a transient increase in thermal latencies that peaked at 30 to 60 min and began to wane 2 h after dosing. These differences were significant at 30 min for all doses

Table 1. Summary of Surgical Outcome of Animals Prepared with Intrathecal Polyethylene and Polyurethane Catheters

Preparation	Material and Treatment	Attempted Catheter Placement	Died Intraoperatively	Complete Recovery	Motor Deficit Post Implant	Failure to Thrive (Euthanasia)
Implant with bolus dosing	Polyethylene	5	0	5	0	0
	Polyurethane	7	0	7	0	0
Implant with pump	Polyethylene + morphine sulfate	16	0	16	0	1
	Polyethylene + 0.9% NaCl	5	0	4	1	0
	Polyurethane + morphine sulfate	4	0	4	0	1
	Polyurethane + 0.9% NaCl	7	0	5	2	0
Implant—closed catheters	Polyethylene	4	1	3	0	0
	Polyurethane	3	0	3	0	0
Implant—pump disconnect	Polyurethane	4	0	4	0	0
	Total	55	0	51	3	2
	Polyethylene	30	1	28	1	1
	Polyurethane	25	0	23	2	1

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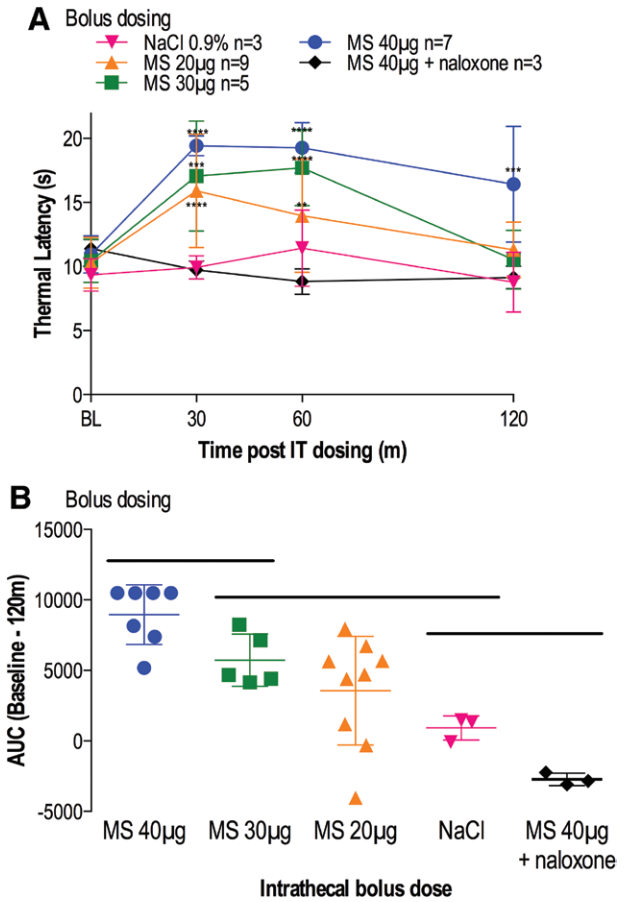

Fig. 1. Intrathecal NaCl and morphine sulfate (MS) dose response. (*A*) Thermal escape latencies (mean ± SD) in guinea pigs plotted *versus* time for groups receiving intrathecal (IT) bolus of 0.9% NaCl, MS (10, 20, or 40 µg in 10 µl), or 40 µg morphine with 10-min pretreatment of 1mg/kg naloxone intraperitoneally. A two-way ANOVA with repeated measures resulted in significance (interaction, $^{***}P = 0.0003$; treatment, $^{***}P = 0.0001$; and time, $^{****}P < 0.0001$). *Post hoc* analysis was performed with Bonferroni multiple comparisons to baseline ($^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$). (*B*) Scatter plot showing area under the curve (AUC; baseline, 120 m) plotted with individual animals by treatment group. Analysis with a one-way ANOVA across treatments yields $^{****}P < 0.0001$. Tukey multiple comparison *post hoc* test comparing all treatments were with significance. Data sets not connected by solid line differ at $^{*}P < 0.05$.

as compared to the preinjection control. Calculation of the AUC for the thermal escape latency was plotted across doses. As indicated (fig. 1B), the highest dose was significantly greater than vehicle. A 10-min pretreatment with naloxone (1 mg/kg intraperitoneal) prevented an increase in thermal latency otherwise observed after a single bolus dose of morphine sulfate (40 µg; fig. 1).

Adverse Events. Bolus intrathecal morphine at doses up to 40 µg had no effect on placing and stepping nor on the pinna and blink reflexes. Biting and/or scratching of the hindquarter were observed after intrathecal injection of morphine 20 µg (2 of 6), 30 µg (3 of 5), and 40µg (3 of 5). These effects were observed by 10 min after injection and were resolved by 30 to 60 min post dosing. No animal receiving vehicle (0.9% NaCl) demonstrated scratching behavior. Animals given a 10-min pretreatment of naloxone 1 mg/kg intraperitoneally followed by intrathecal morphine sulfate 40 µg were observed to bite and scratch their hindquarter, with the effects observed approximately 10 min post dosing. These effects resolved by 30 min post dose. Naloxone pretreatment did not prevent scratching behavior.

Necropsy. At necropsy, all surviving animals displayed catheters located within the intrathecal space with catheter tips at the level of the lumbar enlargement. Distance from the cisterna corresponded with the relative length of the catheter. The 11.5-cm catheter was terminated at the L2–L3 spinal level. Assessment of the spinal distribution of blue dye injected through the L2 catheter at necropsy revealed that the 10 µl injection resulted in a spread of intrathecal dye approximately 2 to 3 cm rostral to the catheter tip (*e.g.*, rostral from L2) and 1 cm caudal to the catheter tip (*e.g.*, caudal to L2).

Phase 2: Chronic Spinal Polyethylene/Polyurethane Catheter Implants

Guinea pigs were implanted with 11.5-cm polyethylene or polyurethane catheters and assigned to one of several treatments: no infusion-externally sealed catheters or pump infusion of vehicle (saline) or morphine. Effects on thermal escape thresholds and incidence of adverse events were assessed periodically and spinal cords harvested after termination of infusion at 14 to 16 days.

Outcome

Of the 43 catheters implanted, 25 were polyethylene and 18 were polyurethane. Of the 25 animals implanted with polyethylene catheters, 23 survived the surgery without motor impairment. Of the two nonsurviving animals, one was euthanized postoperatively for motor impairment and the other died intraoperatively, never recovering from surgery. Of the 18 animals implanted with polyurethane catheters, 16 animals survived the surgery without motor impairment. Both nonsurviving animals were euthanized postoperatively for motor impairment.

Analgesic Activity

In the absence of infusion, animals with polyethylene or polyurethane catheters showed either no change or a modest reduction in thermal escape latency over the 14-day period. Animals with polyethylene catheters and infused with morphine sulfate (4 µg/h or 0.125 µg/h) showed modest increases from baseline on day 3 post implant, although these changes were not statistically significant. These animals had returned to baseline values by day 10 post implant. Animals implanted with polyethylene catheters and dosed with morphine sulfate 12.5 µg/h showed decreases in thermal

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latencies over the 16-day time course, although this was without statistical significance (fig. 2). Animals with polyurethane catheters and infused with morphine sulfate 12.5 μg/h displayed a statistically significant increase in thermal latencies on day 1 and day 3 (fig. 3). These animals returned to presurgical baselines by day 7.

Adverse Events

For the polyethylene catheters, of the four animals dosed with morphine sulfate 12.5 μg/h, two presented with severe scratching on day 3. One animal dosed with morphine sulfate 12.5 μg/h underwent euthanasia on day 10 due to self-inflicted biting lesion to the hindquarter, abnormal righting reflex, and distress. One animal implanted with a polyethylene closed-end (no infusion) catheter was euthanized on day 10 for weight loss and failure to thrive. No other animals receiving test article presented with test article–related untoward effects.

For the polyurethane catheters, of the four animals dosed with morphine sulfate 12.5 μg/h, two animals presented with scratching behavior on day 3, and one animal presented with persistent scratching behavior on day 7. One animal was euthanized on day 7 due to weakness, weight loss, chronic diarrhea, abnormal righting reflex, and abnormal mentation. No other groups presented with test article–related untoward effects.

Histopathology

Forty spinal cords were examined in animals with catheters in place for 16 days. In two animals, the catheter was found in the spinal parenchyma and no score was assigned to these. In four cords (polyethylene-1.25 μg/h morphine, polyurethane-catheter disconnected from pump, polyurethane-catheter disconnected from pump, and polyurethane-12.5 μg/h morphine), evidence of a catheter tract could not be identified in these or adjacent blocks and no score could be assigned. At necropsy, catheters were identified in these animals. While the absence of observations may mean no reaction, it may also reflect the inadvertent loss of the catheter tip reaction during tissue processing. Thus, to be conservative, these four animals were excluded from the analysis. Histopathology was assessed on a scale of 0 to 4 (0: no reaction, 4: severe pericatheter reaction/large space-occupying mass), and all animals were rank ordered overall for severity of pathology. Guinea pig spinal tissues were submitted for grading and ranking for severity of pathology according to the criteria outlined in the previous sentence, and examples of each score can be found in Supplemental Digital Content 2B (http://links.lww.com/ALN/B290). Figure 4 presents scatter plots of the spinal histopathology grade at a level proximal to the catheter tip (mean ± SD). The groups were sorted by catheter material and treatment, and were graded by three independent observers. As noted, scores of 1 to 2 indicate a pericatheter fibrosis, while scores of 3 to 4 indicate progressively increasing pericatheter cellular masses. Figure 5 presents the scatter plot showing the mean rank order assigned by the three independent observers to each of the animals receiving a polyethylene or polyurethane catheter implant with no infusion, NaCl infusion, or morphine (12.5 μg/h) infusion. Observer ranking concordance was calculated, and the intraclass correlation was found to be very high (ICC = 0.9601), with an F value of greater than 0.1 indicating no difference between raters, and a high level of concordance.

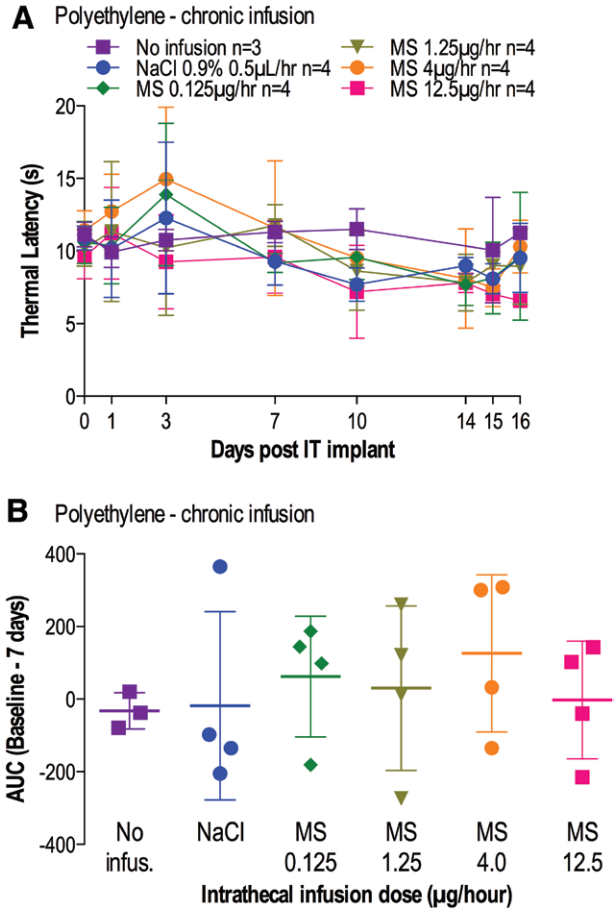

Fig. 2. Thermal escape latency with no infusion or chronic infusion of NaCl or morphine sulfate (MS) with polyethylene catheters. (*A*) Thermal escape latencies (mean ± SD) in guinea pigs plotted *versus* time for groups implanted with intrathecal (IT) polyethylene catheters and receiving chronic infusion of 0.9% NaCl (NaCl), MS (0.125, 1.25, 8.0, or 12.5 μg/h) or no infusion. Results were analyzed using two-way repeated measures ANOVA and show significance (interaction, not significant; treatment, $P = 0.0197$; and time $P = 0.0006$). Bonferroni *post hoc* tests comparing to baseline were without statistical significance. (*B*) Scatter plot showing area under the curve (AUC; baseline, 7 days) plotted with individual animals by treatment group. One-way ANOVA by treatment and Tukey multiple comparisons *post hoc* test were without statistical significance across treatment groups ($P = 0.8812$).

Effects of Polyethylene and Polyurethane Catheters Alone. Three animals were implanted with polyethylene catheters and three with polyurethane catheters. Catheters were sealed at the external ends (*i.e.*, no infusion

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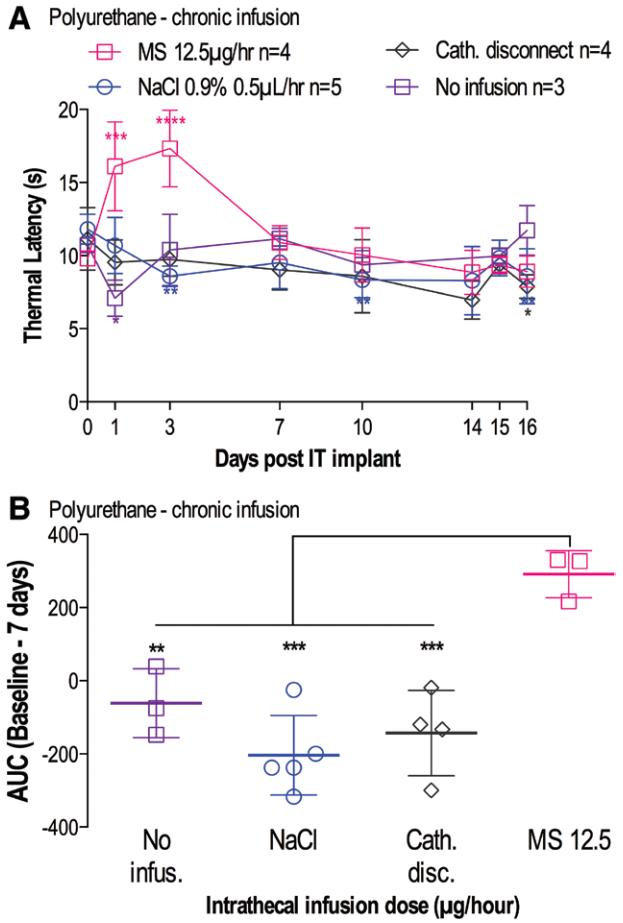

Fig. 3. Thermal escape latency with no infusion or chronic infusion of NaCl or morphine sulfate (MS) with polyurethane catheters. (*A*) Thermal escape latencies (mean ± SD) in guinea pigs plotted *versus* time for groups implanted with intrathecal (IT) polyurethane catheters and receiving chronic infusion of 0.9% NaCl, MS (12.5 µg/h) or no infusion. Group size for animals treated with MS 12.5 µg/h was n = 4 for baseline through day 3 and n = 3 for days 7 to 16. Results were analyzed using two-way repeated measures ANOVA and show significance (interaction, $^{****}P < 0.0001$; treatment, $^{*}P = 0.0266$; and time, $^{**}P = 0.0086$). Bonferroni *post hoc* tests comparing to baseline showed statistical significant increase in latencies for MS 12.5 µg/h on days 1 and 3 ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$). (*B*) Scatter plot showing area under the curve (AUC; baseline, 7 days) plotted with individual animals by treatment group. One-way ANOVA by treatment $P = 0.0002$ and Tukey multiple comparisons *post hoc* test were significant compared across treatment groups ($^{**}P < 0.001$ and $^{***}P < 0.001$).

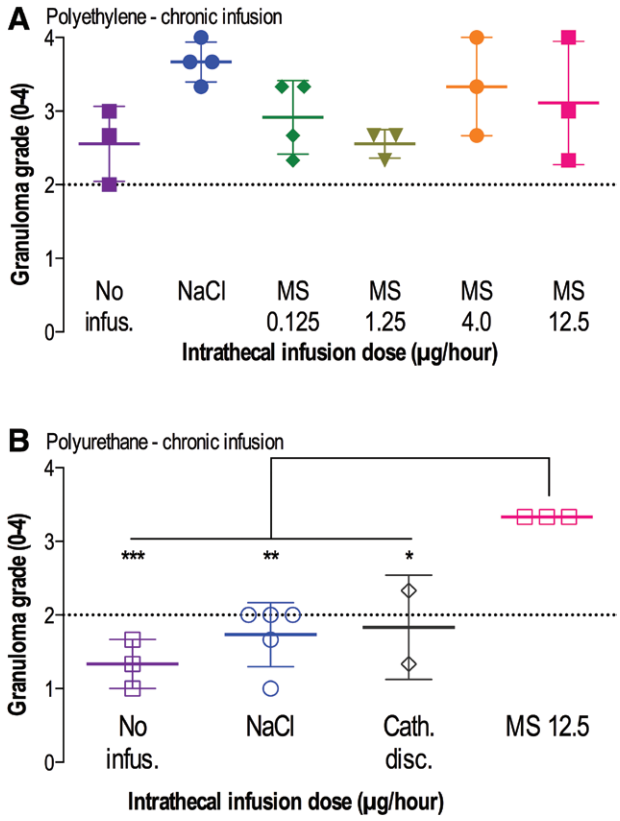

Fig. 4. Pathology scores. Scatter plot presenting the pathology scores (0: no sign through 4: robust space-occupying granuloma) for individual animals presented by treatment group in animals prepared with polyethylene (*A*) or polyurethane catheters (*B*). Note that scores 0 to 2 indicate increasing pericatheter reactions and spinal cord distortion. Scores 3 to 4 (above *horizontal dashed line*) indicate presence of a pericatheter cellular accumulation leading to increasing cord distortion. Grades were assigned based on a standard scoring system by three independent observers blinded to the treatment. Polyethylene: One-way ANOVA ($P = 0.0902$) with Tukey multiple comparison test comparing all treatments, no statistical significance. Polyurethane: One-way ANOVA ($^{***}P = 0.0009$) with Tukey multiple comparison test comparing all treatments ($^{**}P < 0.01$ morphine sulfate [MS] 12.5 *versus* NaCl, $^{***}P < 0.001$ MS 12.5 *versus* no infusion, and $^{*}P < 0.05$ MS 12.5 *versus* catheter disconnect).

was undertaken). The typical picture for all catheters was minimally a thin fibrotic investment of the catheter. Figure 6 presents representative histology at the cervical, thoracic, lumbar, and sacral levels from two animals prepared with polyethylene and polyurethane catheters. As noted in the absence of infusion, there was a minimal reaction, but the polyethylene catheter resulted in a reliably greater indentation of the spinal cord, visibly thicker fibrosis, and, accordingly, higher pathology scores and rankings (figs. 4 and 5).

Effects of Vehicle *versus* No Infusion. Of the six animals receiving no infusion (three polyurethane and three polyethylene), four (three polyurethane and one polyethylene) showed a modest pericatheter fibrosis and no granulomas (scores of 2 or less). In contrast, of the nine animals receiving saline infusion, four (all polyethylene) showed a significant space-occupying mass (fig. 7). The other five animals (all polyurethane) showed modest pericatheter fibrosis and no granulomas (scores of 2 or less). Examining the rank order of saline-treated and no-infusion groups, the order of the mean ranks showed comparative degrees of pathology ranging

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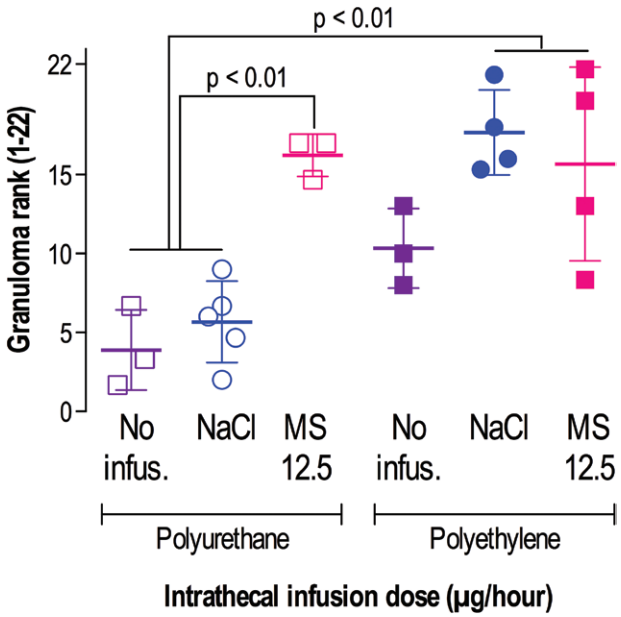

Fig. 5. Rank ordering of pathology. Scatter plot presenting the ranked order of pathology scores (1 to 22) for individual animals presented by treatment group in animals prepared with polyethylene or polyurethane catheters for select treatment groups (no infusion, NaCl, or morphine sulfate [MS] 12.5µg/h). Rankings were assigned from least to greatest pathology by three independent observers blinded to the treatment. Two-way ANOVA (interaction, $P = 0.0091$; treatment, $P = 0.012$; and catheter material, $P = 0.0011$) with Tukey multiple comparison test comparing all treatments showed significance ($P < 0.01$ and $P < 0.001$).

from the least: polyurethane (no infusion) < polyurethane (NaCl) < polyethylene (no infusion) < polyethylene (NaCl). This difference in rank order may reflect the greater degree of spinal indentation observed with and greater stiffness of the polyethylene catheters and/or a leachable material.

Vehicle *versus* Morphine. Of the 16 animals receiving intrathecal morphine with polyethylene catheters, 14 displayed a granuloma score greater than or equal to 2. Of the four animals receiving intrathecal morphine with polyurethane catheters, three displayed a granuloma score greater than or equal to 2; the fourth animal from this treatment group was unable to be scored or ranked due to uncertainty as to catheter tract location. Considering only the morphine-treated groups, the incidence of granuloma as displayed by a pathology score greater than or equal to 2 in polyethylene-catheterized animals is as follows: 0.125 µg/h: 4/4; 1.25 µg/h: 3/4; 4 µg/h: 3/4; 12.5 µg/h: 4/4; in polyurethane catheters, the incidence is 12.5 µg/h, 3/3. Figure 8 displays the results of eight guinea pigs prepared with lumbar polyurethane catheters and that received saline (0.5 µl/h) or morphine 25 mg/ml/0.5 µl/h for 14 days. After perfusion fixation, the cords were submitted for MRI. Based on images, decalcified cords were blocked to perform histopathology at the catheter site (fig. 8). In animals receiving morphine sulfate (25mg/ml; 12.5 µg/h), there is a significant collection of inflammatory cells forming a space-occupying mass that yields progressive degrees of cord compression with maximum girths observed in spinal blocks proximal to the catheter tip and typically extending cranially along the catheter. As indicated, saline resulted in minimal reactions, whereas the addition of morphine led to a prominent intrathecal collection of inflammatory cells after 14 days of infusion.

Examination of Morphine Granuloma Morphology in the Guinea Pig. Examination of mass morphology in the polyurethane animals after 14 days of continuous morphine infusion revealed several common characteristics.

(i) The mass represents a well-demarcated, localized nodular mass, with excessively high numbers of DAPI(+) fibroblast cells inside the mass (figs. 9 and 10). A sharp demarcation indicates the GFAP(+) spinal cord tissue and GFAP(–) granuloma mass. There was little inflammatory filtrate.

(ii) The collection of fibroblasts and inflammatory cells arise from the inner aspect of the duraarachnoid layer (fig. 9).

(iii) The collection of cells constituting the mass surrounding the catheter track was largely composed of fibroblasts compressing the adjacent spinal cord. In spite of the tight apposition of the mass, an intact pia mater could frequently be detected beneath the adjacent mass (fig. 9).

(iv) Evidence of neovascularization in the mass was supported by the presence of endothelial cells (fig. 9).

(v) Collagen staining using Herovici method revealed ongoing collagen formation as evidenced by immature collagen adjacent to the catheter and more mature collagen in the outer layers (fig. 9).

(vi) Cellular composition of the granuloma, aside from fibroblasts, consisted of macrophages and very few lymphocytes (fig. 10). The well-demarcated, localized nodular mass displayed large numbers of DAPI(+) cells inside the mass. Many Iba1+ cells within the mass show morphologic features of monocytes/histiocytes with oblong nuclei. In addition, process-bearing Iba1(+) microglia are found in the spinal cord adjacent to the mass. A sharp demarcation is apparent between the GFAP(+) spinal cord tissue and GFAP(–) granuloma mass. No reactivity for T lymphocytes using the monoclonal T cell receptor antibody (AbD Serotec) was observed in these sections. This observed absence of lymphocytes in the mass was confirmed in the H&E analysis (fig. 9).

(vii) Mast cells could be readily identified in the spinal meninges (fig. 9). Although not systematically quantified, lumbar meninges in the vicinity of the granuloma showed fewer mast cells, as identified by Astra blue staining.

Examination of Morphine-evoked Granuloma Morphology in the Dog. For comparison, two dogs that had been previously studied for granuloma formation after morphine

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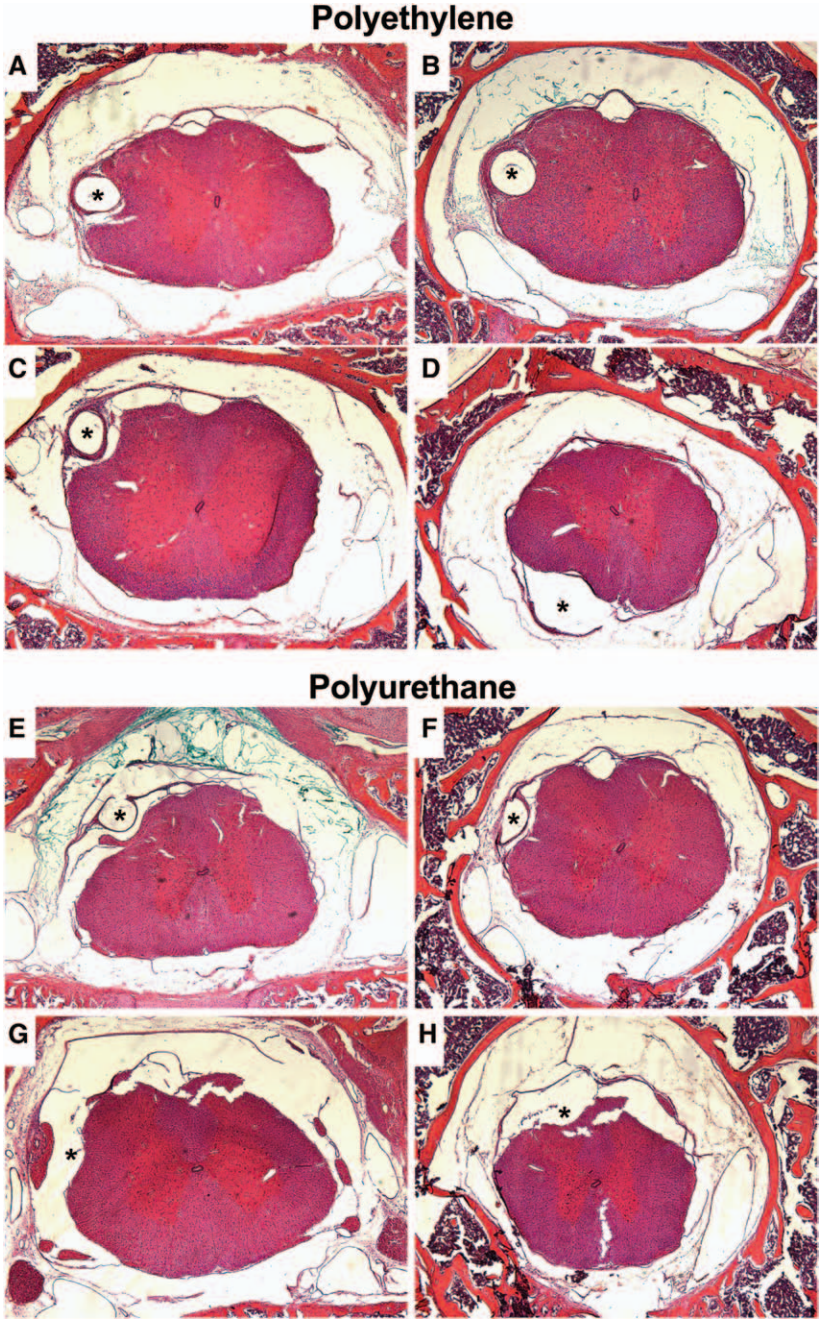

Fig. 6. Cervical, thoracic, lumbar, and sacral histopathology from a polyethylene and a polyurethane animal. Representative cervical (*A*), thoracic (*B*), lumbar (catheter tip level) (*C*), and sacral (below catheter tip) (*D*), hematoxylin and eosin–stained sections from an animal prepared with a polyethylene catheter and harvested at 14 days. Representative cervical (*E*), thoracic (*F*), lumbar (catheter tip level) (*G*), and sacral (below catheter tip) (*H*), hematoxylin and eosin–stained sections from an animal prepared with a polyurethane catheter and harvested at 14 days. No intrathecal infusion was performed. *Catheter profile. Note indentation of parenchyma and more robust pericatheter fibrosis in polyethylene animal (*A* to *D*).

infusion (25 mg/ml at 20 μl/h) in a previous study[9] were examined, and the results from one is presented for comparative purposes (fig. 11). As shown in this animal, the pericatheter mass at the level of the lumbar catheter tip was slightly less than half the diameter of the cord. The observed reaction around the catheter was characterized by an infiltrate throughout much of the subarachnoid space and extending through the dura into the epidural space. The mass was well organized and consisted largely of fibroblasts, with additional macrophages and lymphocytes. Neovascularization was observed as evidenced by endothelial cells in the mass. With the trichrome stain, dense collagen staining was observed around the mass with immature collagen noted proximal to the catheter track and more mature collagen in

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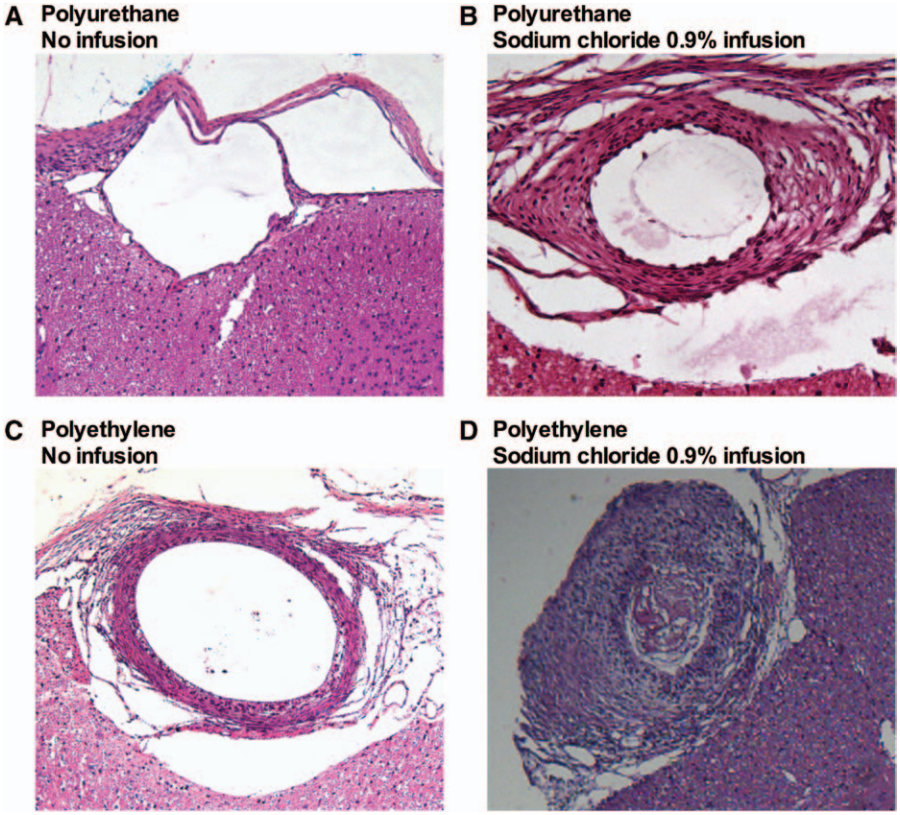

Fig. 7. Polyethylene catheter with or without NaCl infusion. Representative hematoxylin and eosin–stained sections taken at the lumbar level proximal to the catheter tip from animals that had (*A*) a polyurethane catheter with no infusion, (*B*) a polyurethane catheter with 0.9% NaCl infusion, (*C*) a polyethylene catheter with no infusion, and (*D*) a polyethylene catheter with 0.9% NaCl infusion. All animals were harvested at 14 to 16 days after catheter placement.

the outer layers (fig. 11). In canine spinal meninges, mast cells could be readily identified (fig. 11).

Discussion

While intrathecal efficacy studies can be carried out in mice and rats, assessment of safety requires validated models that display pathology anticipated in the human,[27] such as the space-occupying mass produced by continuous infusion of opioids.[28,29] Preclinical work has been limited to large-animal models. While important for the study of pharmacokinetics and drug effects before human use, these models make systematic studies on the biology of spinal drug toxicity difficult to undertake for technical and economic reasons. The current studies had three aims: (i) demonstrate intrathecal catheterization in the guinea pig, (ii) demonstrate utility of the guinea pig as a model of intrathecal opiate drug action, and (iii) determine if the model demonstrates a granuloma pathology with morphine infusion.

Catheter Placement

Using a cisternal approach as employed in the rat, we reliably placed catheters at a distance corresponding to the lumbar enlargement (approximately 11.5 cm in adult guinea pig). Choice of the cisternal approach is predicated on introducing the catheter distant from the lumbar dermatomes where the drug is delivered (catheter tip) and where the innervation of the hind paws (sites of stimuli application) is supplied.

Spinal Catheter

Both polyethylene and polyurethane (with a stylet) catheters could be passed to the lumbar site. Ranking reactions to the catheter revealed that the polyurethane induced a reliably lesser reaction along the track and at the tip. In comparably sized catheters, an evident difference was noted wherein polyethylene resulted in greater parenchymal distortion/indentation. We see the indentation as a sign of the increased radial stiffness of polyethylene *versus* polyurethane catheters. The increased axial stiffness of polyethylene within the flexible spine over time may also lead to a more pronounced reaction at the catheter tip. While long-term tolerability could not be distinguished as, for example, with weight loss or changes in motor function in control animals, the polyurethane catheter was considered to have less impact on spinal morphology.

Effects of Bolus Intrathecal Morphine

Bolus intrathecal morphine resulted in a dose-dependent, naloxone-reversible analgesia and, at high doses, hindquarter biting and scratching. Biting and scratching have been

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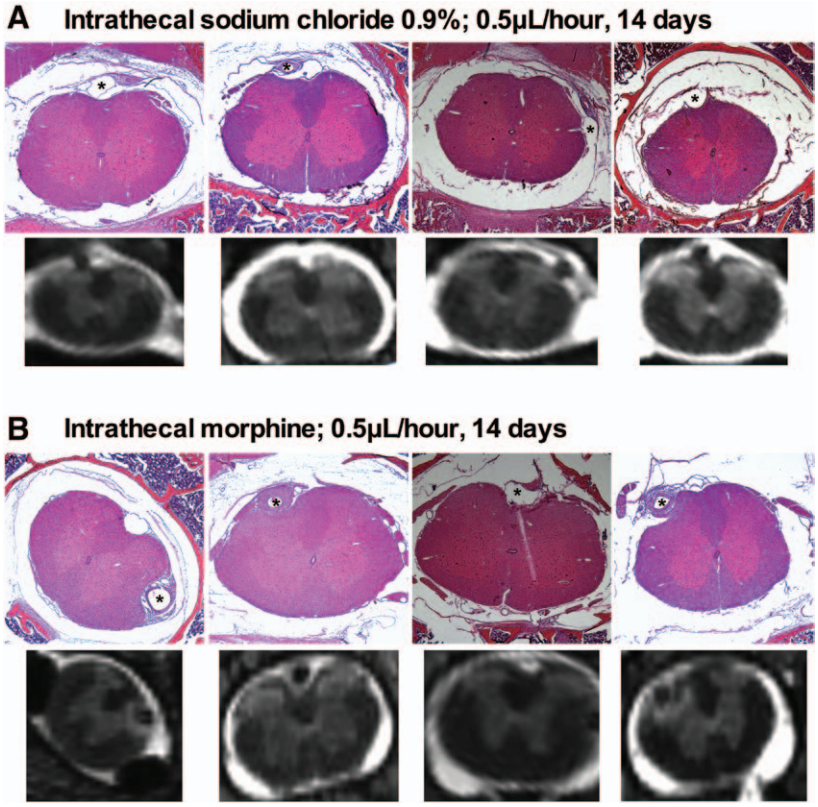

Fig. 8. Polyurethane catheter with NaCl or morphine sulfate infusion and magnetic resonance imaging (MRI). Representative hematoxylin and eosin–stained sections taken at the lumbar level, proximal to the catheter tip, from animals with polyurethane catheters and receiving infusions of saline for 14 days (*A*) or morphine sulfate (25mg/ml at 0.5μl/h) (*B*). All animals were harvested 16 days after catheter placement. For each animal, a postmortem MRI was taken to identify the catheter site and local reaction. *Catheter site.

reported in rats[30–32] and dogs.[33] As in rats, these effects were found to be naloxone insensitive.

Effects of Continuous Intrathecal Morphine Infusion

Infusion of morphine with polyurethane catheters led to an increase in thermal escape latencies that peaked during the first 3 to 5 days but was absent by day 7. This loss of effect is observed in rodents with continuous delivery.[34,35] Unexpectedly, infusion of multiple morphine concentrations using polyethylene catheters had no statistically significant effect even at the highest concentration. The reason for this lack of effect in the polyethylene-catheterized animals is not known. Previous work in rats has reliably demonstrated a dose-dependent analgesia with infusions of a variety of agents.[36,37] As noted in the Spinal Pathology section of the Discussion, however, the most profound histopathologic reactions were observed in animals with polyethylene catheters. Previous work indicated that inflammatory masses alter spinal drug distribution in dogs might lead to a lack of drug effect in relevant spinal segments.[4]

Spinal Pathology

In the majority of work, vertebral columns were removed, decalcified, and blocked. In a number of tissues, cords were submitted for *postmortem* MRI. This *post vivo* identification of the catheter reaction with the catheter *in situ* simplified identification of the level at which the histologic analysis should be focused. This histopathologic analysis revealed robust effects of catheter material and morphine.

Vehicle Effects. Guinea pigs with polyurethane catheters receiving continuous infusions of saline displayed pathology that ranked similarly to that observed in the catheter-only animals and displayed the least evident histologic reactions. In contrast, comparison of catheter reactions with vehicle infusion in polyethylene-catheterized guinea pigs revealed an unexpected reaction that was routinely ranked as being more prominent than in animals treated with catheters without infusion. The vehicle for both polyurethane- and polyethylene-catheterized animals was preservative-free 0.9% NaCl. We note that, to our knowledge, there are no systematic reports, as with the present guinea pig studies, systematically comparing implanted different catheter materials with and without saline infusion.[4,9] As polyurethane catheters showed no difference between saline-treated and noninfused animals, this raises the speculative hypothesis that there may be an eluted substance (leachable) in the polyethylene catheters. This hypothesis requires further study.[38]

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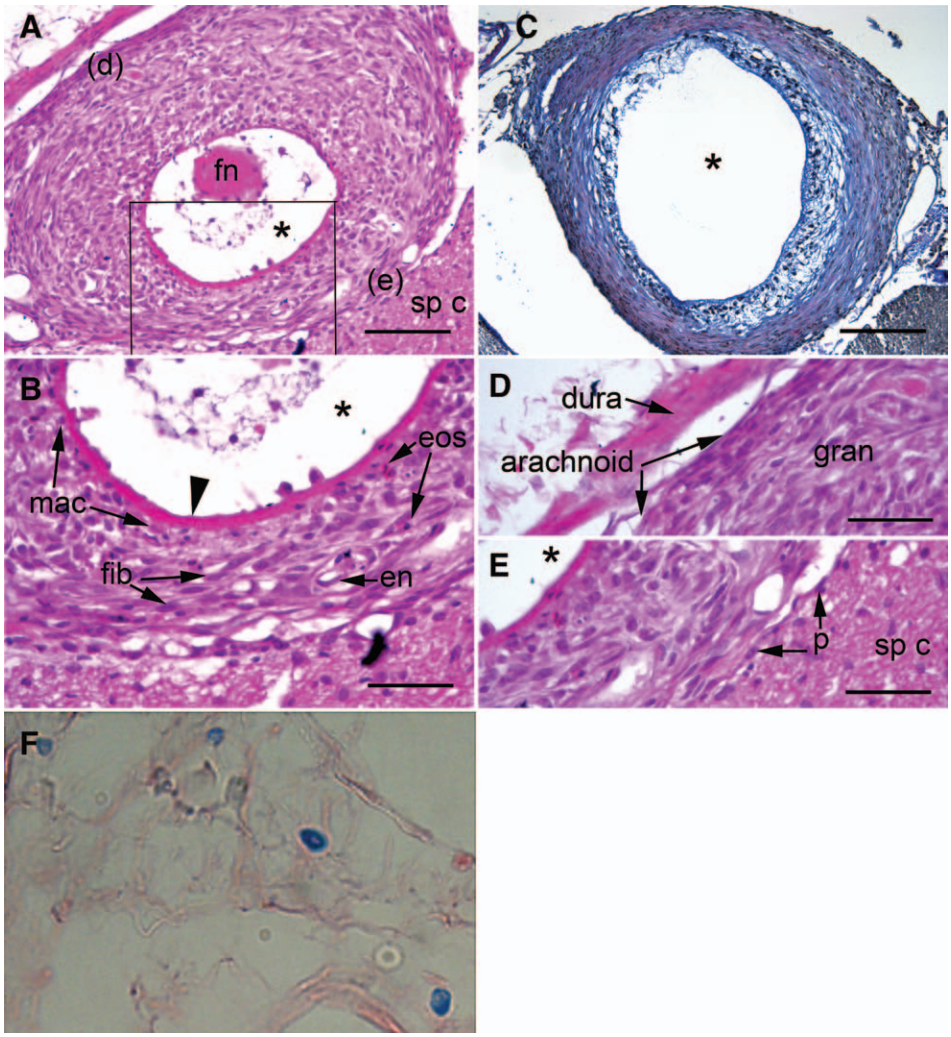

Fig. 9. Guinea pig hematoxylin and eosin (H&E)/collagen/mast cells. Histology from lumbar spinal cord of guinea pig receiving infusions of morphine sulfate (25 mg/ml/0.5 μl/h) for 14 days. (*A*) H&E stain showing subarachnoid inflammatory mass adjacent to catheter (*) and producing local compression of spinal cord (sp c). The catheter site lumen contains fibrin (fn) and a few mixed inflammatory cells. These sections were cut and stained with the surrounding vertebra (not visible in these images) to preserve the pericatheter inflammatory mass and the stain is very eosinophilic due to the decalcification procedure. *Scale bar* = 100 μm. (*B*) Enlargement of the area in the *box* in (*A*). The pericatheter reaction consists of mostly fibroblasts (fib), some macrophages (mac), a few eosinophils (eos), and a few small blood vessels, potentially neovascularization, as indicated by endothelial cells (en). There are few, if any, cells with the morphology of lymphocytes or plasma cells. The inner border of the pericatheter inflammatory reaction (*arrowhead*) most likely consists of a mixture of fibrin and immature collagen. *Scale bar* = 50 μm. (*C*) Herovici collagen stain of the reaction around the catheter site (*) from a different guinea pig showing more mature collagen (*red stain*) in the outer layers compared to the inner layers (*blue stain*, immature collagen). *Scale bar* = 100 μm. (*D*) Higher magnification of the area in (*A*) designated (d). The dura in this section is intact and not infiltrated by inflammatory cells. The inflammatory mass (gran) is entirely within the arachnoid/subarachnoid space. *Scale bar* = 50 μm. (*E*) Higher magnification of the area in (*A*) designated (e). This shows the interface between spinal cord and inflammatory mass. The pia mater (p) is intact and there is no infiltration into the spinal cord (sp c). The catheter lumen (*) is to the left. *Scale bar* = 50 μm. (*F*) Astra blue staining mast cells in the spinal meninges.

Morphine Effects. Intrathecal infusion of morphine at a commercially available concentration (25mg/ml) led to development of granulomas at 14 to 16 days. As noted, these studies do not permit assessment of the concentration dependency of the observed morphine effects as the concentration studies were carried out in the polyethylene-catheterized animals wherein the vehicle resulted in a prominent reactions.

Comparison of the Intrathecal Granuloma in the Guinea Pig versus *Dog, Sheep, and Human*

The current study using polyurethane catheters demonstrated that chronic infusion of morphine, but not vehicle, yields a space-occupying mass that resembled the phenomena observed in other species, including humans.[4,6,9,39]

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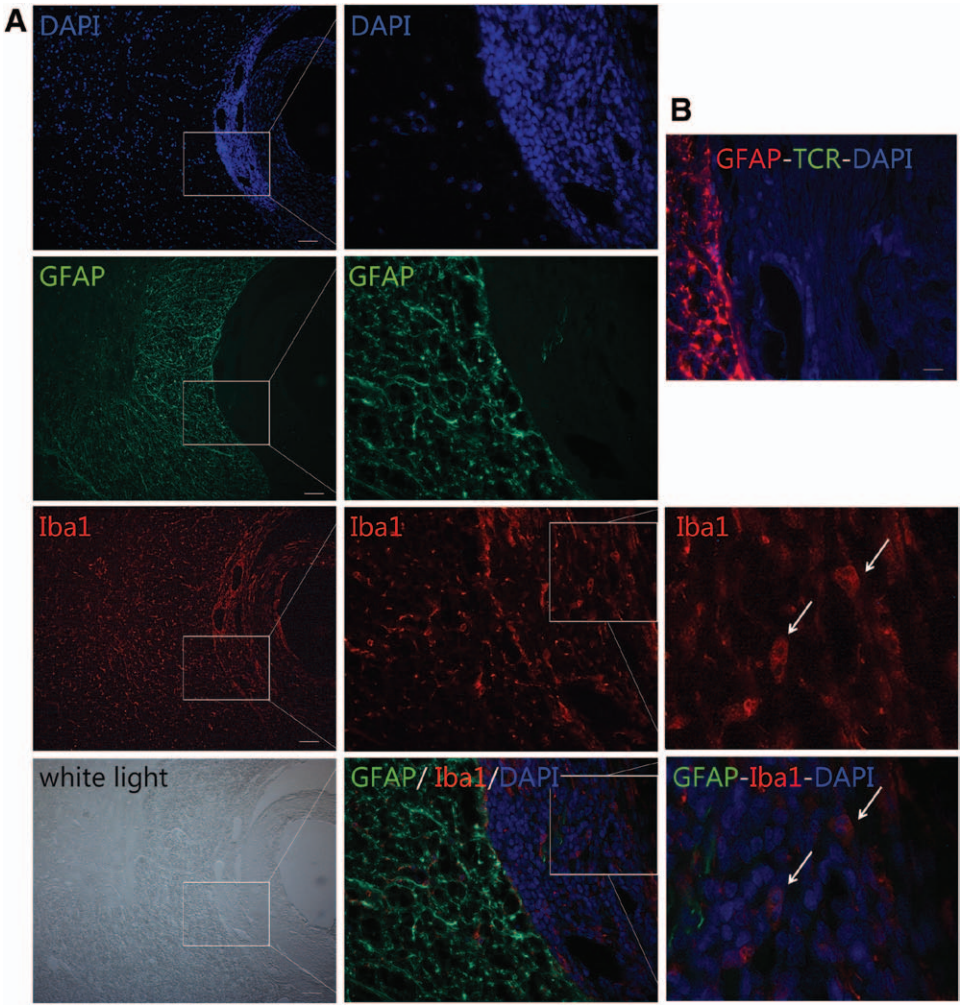

Fig. 10. Guinea pig immunostaining. Monocyte/macrophage immunoreactivity within the granuloma. (*A*) Dual-immunostaining of glial fibrillary acidic protein (GFAP; *green*) and ionized calcium-binding adapter molecule 1 (Iba1; *red*) in guinea pig spinal cord sections exposed to morphine sulfate (12.5 mg/ml/0.5 μl/h) for 14 days. 4′,6-Diamidino-2-phenylindole (DAPI), nuclear fluorescent stain (*blue*). *Arrows* indicate Iba1+ monocytes/histocytes with oblong nucleus inside the GFAP-negative granuloma mass. *Scale bars* = 55 μm. (*B*) Dual-immunostaining of GFAP (*red*) and T cell receptor (TCR; *green*) in spinal cord sections described in (*A*). DAPI, nuclear stain (*blue*). No TCR reactivity is observed in GFAP-negative granuloma mass. *Scale bar* = 25 μm.

Time Course. The current study only examined one time point permitted by use of a 2-week implantable 0.5μl/h pump. In large-animal preclinical investigations, the majority of work has examined tissues at intervals of approximately 28 days.[4,6,9] In the dog, serial MRIs, however, revealed the appearance of an identifiable mass at periods as early as 10 to 14 days after initiation of morphine infusion.[7]

Mass Size. The preclinical MRI work in dogs demonstrated a progressive growth of these masses over time,[7] where, by 24 to 28 days, the cross-sectional diameter may approximate half of the spinal cord.[4,6,9] As the size of the granuloma observed in these studies relative to spinal cord diameter was less than those noted in dogs and sheep, this raises the possibility that larger granulomas in the guinea pig may be observed with a longer infusion exposure.

Granuloma Microanatomy. The guinea pig morphine-induced granuloma has several defining properties that correspond with the canine granuloma. (i) It originates from the dura-arachnoid and not from the underlying pia or spinal cord. (ii) As previously reported,[4,38] the mass is composed of an inner core adjacent to the catheter track consisting of fibroblasts and an outer band displaying fibroblasts and inflammatory cells. (iii) Staining emphasizes the progressive increase in collagen, with staining of mature collagen observed in the outer margin of the mass and less mature collagen proximal to the catheter. (iv) Neovascularization is present within the mass. In humans, the picture is less well characterized, with pathologic findings typically being necrotic tissue, connective tissue, and an inflammatory infiltrate consisting mainly of mononuclear cells.[39]

Study Limitations

The current study demonstrated an effect of intrathecal morphine in the guinea pig, producing a space-occupying mass.

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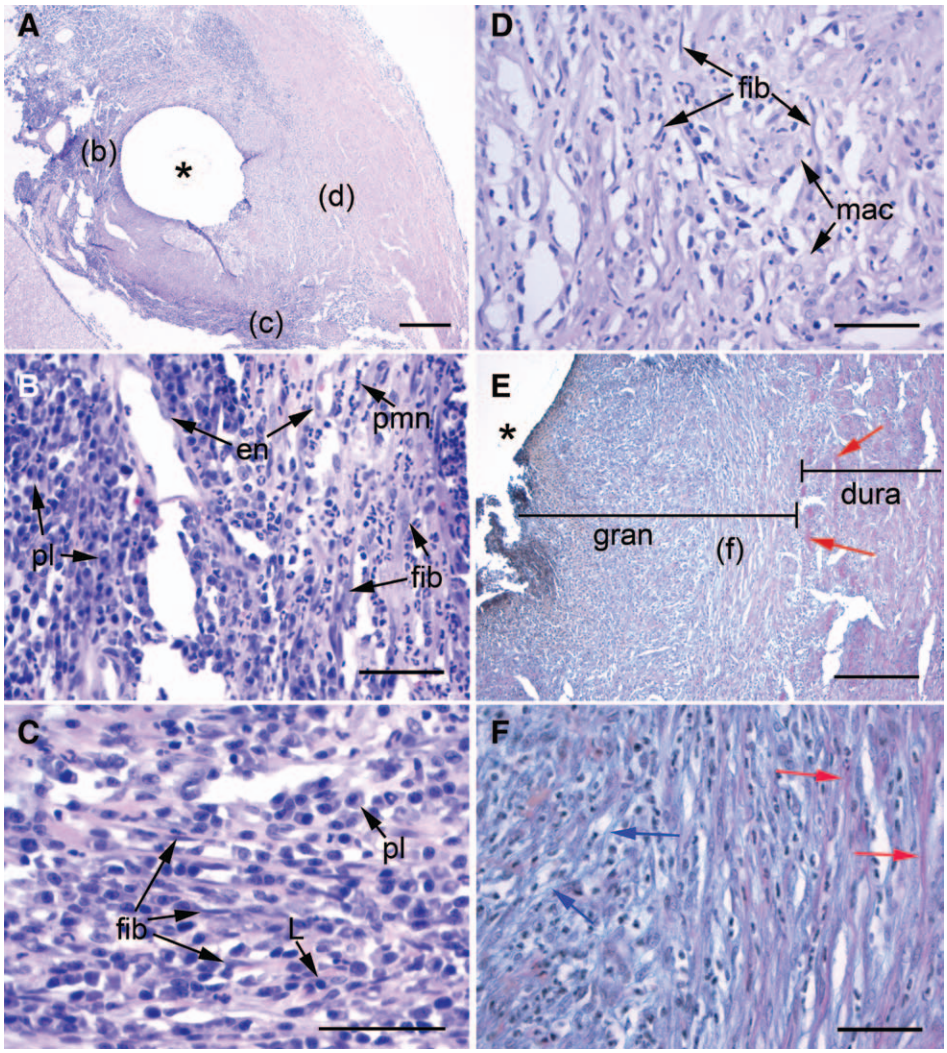

Fig. 11. Dog hematoxylin and eosin (H&E)/collagen/mast cells. Histology from the lumbar spinal cord of a dog that received infusions of morphine (25 mg/ml/0.5 ml/day) for 28 days. (*A*) Low-power view of the catheter site (*) and pericatheter inflammatory reaction stained with H&E. This animal has a severe inflammatory reaction, with infiltrate throughout much of the subarachnoid space and extending through the dura into the epidural space. The areas designated (b), (c), and (d) are shown at higher magnification in (*B*), (*C*), and (*D*). *Scale bar* = 300 μm. (*B*) The inflammatory reaction close to the catheter consists of abundant polymorphonuclear cells (pmn), plasma cells (pl), and some lymphocytes and macrophages (not marked in this image), intermixed with fibroblasts (fib). Reactive endothelial cells (en) suggest neovascularization. *Scale bar* = 50 μm. (*C*) Further away from the catheter, there are fewer polymorphonuclear cells, but still abundant plasma cells (pl) and some lymphocytes (L) intermixed with fibroblasts (fib). *Scale bar* = 50 μm. (*D*) The inflammatory reaction extends to and the cell in this location consists predominantly of fibroblasts (fib) and macrophages (mac). *Scale bar* = 50 μm. (*E*) Herovici collagen stain. Medium power view of the pericatheter reaction in a location similar to that labeled (d) in image (*A*). The catheter site (*) is on the left, dura on the right. Dense bands of mature collagen (*red stain*, examples at *red arrows*) are seen in the dura. The collagen bands in the dura are separated by inflammatory infiltrate. The inflammatory reaction on the *left* (gran) is within the arachnoid/subarachnoid space. The area designated (f) is shown at higher power in (*F*). *Scale bar* = 200 μm. (*F*) The region of the inflammatory reaction furthest from the catheter has more mature collagen (*red stain*, examples at *red arrows*) than the region closer to the catheter (*blue stain*, examples at *blue arrows*). *Scale bar* = 50 μm.

As noted, the mass volume relative to spinal cord was small compared to sheep and dog. However, we speculate that this is because of the 14- *versus* 28-day exposure. The study only examined morphine. It would be of value to demonstrate the effects of other granuloma-inducing opiates (*e.g.*, hydromorphone) in contrast to opiates that have not been shown to yield granulomas (*e.g.*, fentanyl and alfentanil).

Proposed Mechanisms of Granuloma Formation

Canine studies indicate that the morphine-evoked mass is independent of an opioid receptor.[8,9] We hypothesized that mast cells may be an intermediate link in granuloma formation given their opioid receptor–independent degranulation by morphine and other granuloma-producing opioids and the inhibitory effects of a mast cell stabilizer.[9] Meningeal mast

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cells are indeed present in guinea pigs and dogs.[14,15] Interestingly, the common pathology in all species is the massive proliferation of fibroblasts and the exuberant deposition of collagen that continues over time as the morphine is delivered, leading to a mass of progressively increasing size and structural organization. This picture is consistent with two potential pathways by which morphine could stimulate fibroblast activity. First, mast cell degranulation enhances fibroblast chemotaxis. Tryptase and chymases, common products of mast cell degranulation, stimulate collagen messenger RNA transcription.[40,41] Second, morphine causes proliferation of kidney fibroblasts,[42] suggesting the role of morphine in renal interstitial fibrosis. Similar effects have been identified with lung fibroblasts, where tryptase induces lung fibroblast proliferation *via* protease-activated receptor 2, suggesting that morphine-induced tryptase release from mast cells may play a role in the fibroblast proliferative response and scarring observed in chronic lung disease.[43] Several convergent observations are also of interest: (i) inhibition of tryptase reduces fibroblast chemotaxis in dermal fibroblasts[40]; (ii) inhibition of mast cell degranulation leads to a *decrease* in scar formation in skin wound healing[44]; and (iii) blocking mast cell degranulation or the use of mast cell–deficient mice reduces fibrosis.[45,46] Our speculative hypothesis is that morphine stimulates pericatheter fibrosis through either a direct effect on meningeally derived fibroblasts and/or by degranulation of mast cells that release proteases initiating fibroblastic activity and collagen deposition, leading to the aberrant fibroblastic response, *e.g.*, the granuloma is akin to a hypertrophic or keloid scar. We believe that the guinea pig model will be an appropriate small-animal model to assess the merit of these hypotheses.

Conclusions

The current study suggests that the guinea pig with chronic intrathecal catheters can be employed to define mechanisms in the development of space-occupying intrathecal masses and thus suggests a small-animal model for certain investigations focusing on the pathology of spinal drugs.

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Competing Interests

The authors declare no competing interests.

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Address correspondence to Dr. Yaksh: Department of Anesthesiology 0818, University of California, San Diego, 9500 Gilman Dr. (CTF C-312), La Jolla, California. tyaksh@ucsd.edu. 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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