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Characterization of Effect of Repeated Bolus or Continuous Intrathecal Infusion of Morphine on Spinal Mass Formation in the Dog

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Abstract

BACKGROUND.—We determined whether intrathecally delivering the same daily dose of morphine (MS) at a fixed concentration of 25 mg/mL by periodic boluses versus continuous infusion would reduce intrathecal mass (IMs) formation in dogs.

METHODS.—Adult dogs (hound cross, n = 32) were implanted with intrathecal catheters connected to SynchroMed II infusion pumps. Animals were randomly assigned to receive infusion of 0.48 mL/day of saline or MS dosing (12 mg/day at 25 mg/mL) as boluses: x1 (q24h), x2(q12h), x4 (q6 hr) or x8 (q3 hr) given at the rate of 1000 μL/h), or as a continuous infusion (25 mg/mL/20 μL/hour).

RESULTS.—With IT saline, minimal pathology was noted. In contrast, animals receiving morphine displayed spinally compressing durally derived masses with the maximal cross-sectional area being greatest near the catheter tip. Histopathology showed that IMs consisted of fibroblasts in a collagen (type 1) matrix comprised of newly formed collagen near the catheter and mature collagen on the periphery of the mass. The rank order of median cross-sectional mass area (mm^2) was: Saline: 0.7 mm^2; x2: 1.8 mm^2; x4: 2.7 mm^2; x1: 2.7 mm^2; x8: 4.2 mm^2; Continuous: 8.1

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Authorship statement:

In-life phase of this study was performed at Medtronic under the direction of Medtronic employees (three Medtronic scientific research staff: Drs. Keith Hildebrand and Linda Page and Tina Billstrom). Detailed histopathology and mass assessments were performed independently by Dr. Yaksh and Joanne Steinauer at the University of California, San Diego in a blinded fashion. Kelly Eddinger performed the bench diffusion studies and calculations. Drs. Yaksh, Page and Hildebrand and Ms. Billstrom analyzed the data and prepared the manuscript to be submitted for consideration for publication. Dr. Yaksh served as the corresponding author. Dr. Yaksh has performed separate studies under a laboratory service agreement between Medtronic and the University of California, San Diego.

Conflict of Interest statement:

Drs. Keith Hildebrand and Linda Page and Tina Billstrom are Medtronic employees. Dr. Yaksh, Joanne Steinauer, and Kelly Eddinger are employees of the University of California. Dr. Yaksh has collaborated with Medtronic on other studies funded by laboratory service agreements. He has no other benefits deriving from Medtronic.

Prior Presentations:

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mm^2, with statistical difference from saline being seen with continuous ($p < 0.0001$) and x8 ($p<0.05$). Bench studies with a two-dimensional diffusion chamber confirmed an increase in dye distribution and lower peak concentrations after bolus delivery versus continuous infusion of dye.

CONCLUSIONS.—Using multiple bolus dosing, IMs were reduced as compared to continuous infusion, suggesting relevance of bolus delivery in yielding reduced intrathecal masses.

Keywords

intrathecal drug; intrathecal granuloma; intrathecal pump; morphine; bolus delivery

INTRODUCTION

Following initial characterization of the analgesic actions of intrathecal (IT) opioids in animals,[1] IT delivery was widely initiated in humans for management of acute pain.[2] Implementation of chronic indwelling IT catheters with implantable pumps permitted routine continuous delivery of spinal opioid analgesics.[3] Aside from issues pertinent to opioid receptor activation (respiratory depression, sedation, urinary retention), an unanticipated consequence of chronic IT morphine, which first appeared in 1991,[4] was that IT infusion of a variety of mu opioids resulted in neurological signs secondary to local spinal cord compression[5,6]. Preclinical work in canine,[7–9] sheep[10] and guinea pig[11] IT infusion models emphasized that compression resulted from a fibroblast-collagen matrix based mass arising in a time-dependent fashion from the meninges adjacent to the delivery site.[7] The overall incidence of this problem has been estimated, based on neurological signs, to be approximately 0.1% in a population of approximately 13,000 IT pump patients,[12] although estimates in select populations have been as high as 43%.[6] An alternate perspective is that amongst physicians who have a patient with a mass, only 66% reported the patient as displaying neurologic signs. As the diagnosis of spinal masses is typically based on neurological signs, the risk of occult spinal masses is thus likely to be greater.[6]

Factors governing IT opioid-induced mass generation has been a point of particular interest. In humans, the incidence of spinal masses is closely associated in time with implementation of high infusate concentrations of morphine. Prior to 1990, concentrations were typically 10 mg/mL.[13] In 1991, 25 years after the first infusions, the first clinical report of a spinal mass was observed in a patient receiving 20 mg/mL morphine.[4] Since that time there have been over 20 case series of granulomas and the common factor is the use of high infusate opioid concentrations delivered at slow rates.[5,6] Of note in the dog, 12 mg/mL/day results in a high incidence of intrathecal masses.[7–9,14,15] However, we have previously shown that a daily bolus IT injection of 10 mg/mL/day for 28 days in the canine model does not produce such pathology.[16] This led to the speculative hypothesis that bolus delivery may enhance local redistribution and reduce the local concentration to which the tissue near the catheter delivery site would be exposed. We showed that IT infusion in the dog model of a mass-inducing dose at a lower concentration, i.e., greater infusion volume, did not result in a granuloma.[8,9] These results led to the present study, where we compared the intrathecal mass producing effect of the same daily total dose of morphine given daily over 28 days as a continuous infusion versus when given in 1, 2, 4 or 8 divided boluses, all compared against the effect of IT infusion of saline (0.9% preservative-free). Here we report that delivery of a

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standard daily dosage in a divided dose protocol significantly reduced the incidence and severity of IT mass formation otherwise observed with continuous IT infusion of morphine.

MATERIALS AND METHODS

Study approval.

All studies described here were conducted at the Physiological Research Laboratories (Division of Medtronic, PLC., Minneapolis, MN, USA) under protocols approved by the Medtronic Institutional Animal Care and Use Committee.

Subjects.

Male and female hound cross dogs were purchased from approved USDA Class A licensed dealers at 9–13 months in age and weighing 23.0–35.2 kilograms, were individually housed in open-top stainless steel pens that met space/weight recommendations in The Guide for the Care and Use of Laboratory Animals with either epoxy-coated concrete floors or plasticol-coated expanded metal raised floors and were given *ad libitum* access to food and water.

Surgical preparation.

For all animals, the antibiotic Cefazolin (22 mg/kg IV or SQ) was given before surgical incision and every 2 h during surgery with a final dose given during surgical recovery. After pump implant, IT saline animals were given Clavamox (14 mg/kg P.O. BID day 1–10); animals infused with IT morphine were given Primor (55 mg/kg P.O. SID day 1 and 27.5 mg/kg P.O. SID days 2–10). Each animal (receiving intrathecal saline or intrathecal morphine) was started on diphenhydramine prior to surgery to limit the side effects of morphine-induced pruritus. Dosing was 50 mg P.O., TID in the morphine animals which was continued through the duration of the study. All dogs received acepromazaine (0.045–1.0 mg, IM) and were sedated with propofol (4–6 mg/kg IV to effect). After endotracheal intubation, anesthesia was maintained under mechanical ventilation with 1.0–3.0% isoflurane and O_2. Intraoperatively, body temperature was maintained with an underbody heating pad, and the animals were continuously monitored for oxygen saturation, end tidal CO_2 and heart and respiratory rates. Surgical areas were shaved and prepared with chlorhexadine scrub and solution. Using aseptic technique, the cisterna magna was exposed by combined blunt and sharp dissection. The dura was exposed; through a small incision (1–2 mm), the intrathecal catheter (8709SC silicone catheter: 0.5 mm ID/1.4 mm O.D, 6 side orifice with a closed tip) was passed via fluoro-guided assistance from the cisterna magna (CM) to L2-L3 segments (approximately 40–42 cm) and then tunneled subcutaneously to the thorax for connection to a subcutaneous infusion pump (Synchromed II. Model 8637; Medtronic Inc,) with either a 20 or 40 mL drug reservoir) filled with either saline or morphine sulfate. Fluoroscopic images were taken to document catheter path and tip location. A single dexamethasone sodium phosphate dose (0.25 mg/kg, I.M.) was administered to lessen the acute inflammation reaction induced by the surgery and to provide adjunctive post-surgical analgesia. Upon closure of the incision, isoflurane was discontinued, and the animal was recovered. Upon closure of the incision, isoflurane was discontinued, and the animal was recovered. To manage post-operative pain, IT saline-treated animals received Carprofen (4 mg/kg PO, IM or SQ SID days 1–4) and Buprenex (1

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mL (0.3 mg/mL) IM, SQ TID days 1–3 AM dose). Animals receiving IT morphine were administered morphine (0.5 mg/kg IM or SQ TID day 1- day 4 AM dose) and Carprofen (4 mg/kg PO, IM, SQ SID days 1–4). Buprenex was not used in the animals receiving IT morphine to avoid the risk of withdrawal.

In-Life

Drug Delivery.—Administration of morphine sulfate (Infumorph®, 25 mg/mL) via the infusion pump began approximately 4–7 days postoperatively to allow recovery. Infusions of intermittent boluses at rates of 1, 2, 4, or 8 boluses per day were given via the infusion pump; the pump was programmed to deliver the minimum flow (2 μL/h) between boluses. Boluses were programmed to be delivered at the fastest rate possible with the pump (1 mL/h). Boluses were slowly titrated upward (bolus frequency held constant as dictated per group assignment, bolus amplitude increased) every 3–4 days until the targeted dose (approximately 12 mg/day) was reached to allow the animals to acclimate to the supratherapueutic dose. The titration took approximately 14 days. Infusions continued for 28 days after initiation of the highest planned dose. Following euthanasia (after 28 days at the planned infusion rate and dose), the animals were transferred to necropsy for gross and microscopic assessment for inflammatory masses in the IT space.

Behavioral Observations.—Daily observations included assessment of appetite, urine and fecal output, arousal, muscle tone, and motor coordination. Neurologic examination included but was not limited to assessments of gait, muscle tone, posture, cranial nerve and limb reflexes, and response to stimulation. For analysis, specific observations (see listing in SDC table 1) were noted as present or absent in the initial dose-titration phase (days 0–14) and separately in the maintenance-dosing phase (days 15–43).

Treatment of adverse events.—In the course of these studies, adverse events were observed. According to protocol, adverse clinical signs were addressed by pharmacological treatments, temporary adjustment of dose, or study termination for a given animal. The decision tree for addressing adverse events are given in Yaksh SDC Figure 1. During the study, if adverse events were noted that were considered by the veterinary staff to be unmanageable and unacceptable in an animal, that animal was humanely euthanized.

Post-Mortem

Pumps were removed, and methylene blue dye was injected into the catheter to assess for leakage, to note catheter tip location and to confirm catheter patency. The spinal cord was exposed by dorsal laminectomy of the vertebral column. The spinal cord tissues (either retained within the ventral portion of the vertebral canal or removed from the canal) including the catheter tip location were collected and placed in 10% neutral-buffered formalin. After fixation, cross-sections were collected from 1) two cervical spinal cord segments (C2, C6/C7), 2) one mid-thoracic segment (T6-T9), and 3) lumbar cord and dura mater segments (L1, L2, L3, and L5/L6) that ensured that the tissue sample had dura matter proximal to the catheter side ports. Samples were placed in labeled histology cassettes for processing and embedding in paraffin. Each paraffin-embedded tissue block was sectioned at 4–6 microns. Tissue sections were mounted onto histology glass slides. Slides were stained

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with hematoxylin & eosin (H&E). Additional stains included Herovici and Picro-Sirius Red (see [11] for detail of protocol for collagen). When viewed under polarized light, thick (yellow-orange birefringence) and thin (green birefringence) collagen fibers can be identified, respectively, of Types 1 and 3 collagen. Brightfield images were taken of each H&E-stained section using an Olympus BX51 microscope (Olympus America Inc., USA) and Optronics MagnaFire—SP Digital Camera Systems.

Intrathecal Mass Analysis.

To estimate the cross-sectional area of the granuloma, representative transverse sections were taken at the level of the largest girth of the intrathecal mass (typically at or near the lumbar catheter tip) and stained with hematoxylin-eosin. Without knowledge of treatment, the cross-sectional area of the mass and the spinal cord was manually outlined using Image-Pro Plus 5.1 software (Acton Manufacturing Center c/o Media Cybernetics, Inc., Acton, MA) to measure area of the outlined pixels. Pixel count was converted into mm^2.

Bench Assessment of Diffusion.

To assess the effects of infusion delivery protocols (continuous vs. bolus) on the diffusion profile from the clinical-grade catheter (Model 8709SC) employed in these studies, a benchtop two-dimensional diffusion chamber was employed. The model 709SC catheter had 3 paired orifices of approximately 0.34 mm each. A 20 cm clear plastic chamber was filled with saline (0.9% NaCl) to a depth of 3 mm. The catheter was placed in the middle of this volume under the fluid and above the bottom of the chamber. An even backlit illumination was provided with a diffuser plate and the system was continuously imaged with a Nikon camera (Cool pix,16 megapixels). The catheter (Medtronic, MN) employed in the in vivo studies (50 cm in length) was connected to a Medtronic SynchroMed II pump. All components were at the same height to avoid siphon pressures. The catheter was back filled with methylene blue dye (0.5%) in isotonic saline with care taken to avoid air in the system. The pump was programed to deliver 3.3 μL as a continuous infusion at 20 μL/h (0.33 μL/min) for 10 minutes or 3.3 μL as a bolus (e.g. at 1000 μL/h = 16.7 μL/minute). For the bolus, the time to assess diffusion was 10 minutes after the start of the delivery bolus. The 3.3 μL volume was chosen to avoid interference by the limited-volume chamber on dye diffusion. For analysis of each diffusion run, two baseline measures were taken: i) Back ground intensity (e.g. reading prior to the initiation of infusion); and, ii) The distribution of dye density associated with the dye-filled catheter was measured prior to initiation of infusion. For plotting and analysis, dye density was expressed as the change above back ground (relative dye density), e.g. 0 = background (signal in the absence of dye). For plotting, the dye density associated with the width of the dye filled catheter in each run was subtracted from the plot. For quantitative analyses, dye density was plotted along a line placed perpendicular through the catheter at the port proximal to the pump. Pixel density was plotted as a function of distance from the external wall of the catheter. using the software package ImageJ 1.47v (National Institutes of Health, Washington DC). The mean and SEM of the pixel density vs. distance form catheter wall was then plotted. From this best fit line, the peak height and the intercept with “0”density was calculated. In addition, each individual run was examined for peak density and the distance from the catheter wall where the dye

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density fell to zero was determined. Here the distance between left and right sides of the catheter where the dye density declines to background of the signal was calculated.

Statistics

Group sizes in this study were five animals per group. This group size was based on previous work in the canine intrathecal infusion model, where statistically significant changes in mass size for continuous intrathecal morphine vs. intrathecal saline could be shown at $p < 0.01$ using a nonparametric analysis with five animals/group.[15] Comparisons across treatment groups for mass size employed the nonparametric Kruskal Wallis test with *post hoc* comparisons to the saline treatment group using Dunn's multiple comparison test. Group comparisons having critical values corresponding to $p<0.05$ were considered statistically significant. To compare the incidence of adverse events across groups, a Chi square analysis was undertaken. All analyses employed the GraphPad Prism software package (v.4.0c for MAC OS X; GraphPad Software, San Diego, CA).

RESULTS

Morbidity.

A total of 32 animals were implanted; 2 were replaced so that each treatment group contained 5 animals. One animal (339609:x1 bolus) was terminated early (less than 7 days after initiation of infusion) as requested by the veterinary staff as it was symptomatic (ataxic, arching back, pruritus, not eating); this animal was replaced by animal 340620. No mass was observed in animal 339609. Animal 339808 (x2 bolus) completed the study but had an infection/mass near the catheter tip and was excluded from further analysis; this animal was replaced with 340625. Two animals (339615:x2 bolus and 339818: x4 bolus) were sacrificed early (14 days of titration + 10 days of maintenance) as requested by the veterinary animal care staff as they displayed significant clinical signs (ataxic, pruritic, hypersensitive) but they were not excluded from analysis. No masses were detected in either of these animals.

Catheter Placement.

At necropsy, catheter tips were typically located at the L2-L3 spinal level. Dye injection made at sacrifice revealed intrathecal dye present near the catheter tip in all catheters; no catheter leaks or obstructions were evident.

Behavioral and Neurological Observations.

Previous work had shown that delivery of 12 mg/day of morphine sulfate, but not saline, was poorly tolerated in the opioid-naive dog.[7] The nature and incidence across treatment groups of adverse observations during the initial titration phase and the later maintenance-dose phase are provided in SDC Table 1. Biting and scratching at dermatomes proximal to the catheter tip and hyper responsiveness to light touch applied to the flanks and back were the most commonly noted adverse events in all dosing groups receiving morphine, with the exception of the saline-treated animals. As per protocol, animals displaying these behaviors received therapeutic interventions to lessen the behavioral state according to the decision tree presented in SDC Figure 1. The number of animals displaying adverse behaviors in each treatment group was numerically higher in the maintenance-dosing phase compared to the

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dose-titration phase, but a Chi square comparison of the incidence of chewing, tactile sensitivity and scratching was $p>0.05$, respectively, for each clinical sign. Though not systematically assessed, animals administered 1 or 2 boluses/day appeared to display more intense responses to each injection during the initial dose-titration phase as compared to animals that received 4 or 8 boluses per day or continuous infusion. As documented in SDC Table 1, mild-to-moderate hind limb motor dysfunction, mild hyperreflexia and hind limb stiffness was frequently observed in morphine-treated animals at the time of euthanasia.

Intrathecal mass

Figure 1 displays typical gross pathology of an animal receiving continuous intrathecal infusion for 28 days of morphine (12 mg/mL at 0.48 mL/day). The gross dissection shown in Figure 1A reveals the distal end of the intrathecal catheter being embedded in an opaque mass that was approximately 2–3 cm in length with evident adhesion to the adjacent meninges. Figure 1B presents the associated microscopic pathology of this spinal cord and reveals a large space-occupying mass that produces a prominent distortion of the spinal cord parenchyma.

Effects of bolus vs. continuous intrathecal delivery.

Figure 2 presents the respective lumbar histopathology in sections taken near the catheter tip in animals receiving 28-day infusions of: A) IT saline (0.48 mL/day), B) continuous IT infusion of morphine (12 mg/0.48 mL/day) and C) IT infusion of morphine (12 mg/0.48 mL/day) at 1000 μL/h delivered in four equally divided infusion episodes at 6-hour intervals. As shown in Figure 2A, systematic examination revealed that the IT catheter in saline-treated animals resulted in a minimal reaction to the catheter at sites near the catheter tip with minimal parenchymal distortion in all animals with modest spinal cord compression reflecting the cross sectional area required by the physical size of the catheter (N=5). In Figure 2B, the histopathology from five animals receiving continuous IT infusion of morphine resulted in a local thickening of the meninges and a highly evident, space-occupying IT mass. In Figure 2C, the x4 bolus delivery of the same daily morphine dose and concentration resulted in an evident minimization of the intrathecal mass response. The largest compression was noted in animal C2. Figure 3 presents the scatter plots for each animal in the 6 treatment groups: A) for the cross-sectional area (mm^2) and B) the percent of the cross-sectional area of the mass vs. the total cross-sectional area of the spinal cord in that section. For analysis, the cross-sectional area of the catheter profile, was subtracted from the IT mass cross-sectional area. The rank order of median mass cross-sectional area from smallest to largest by treatment was:

Saline:0.72 mm^2; x 2 (q 12 hr) : 2.23 mm^2; x 4 (q 6hr) : 2.66 mm^2, x 1 (q 24 hr) : 2.68 mm^2; x 8: 4.22 mm^2; Continuous: 8.06 mm^2. There was a highly significant main effect observed following a non-parametric one-way ANOVA of cross-sectional area of the IT mass and treatment, with statistical differences from saline being seen with continuous ($p <0.0001$) and x 8 ($p<0.05$).

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Histological examination of IT mass.

Examination of mass morphology in animals receiving IT morphine revealed several common characteristics. The mass was a localized nodular mass which served to markedly compress the adjacent spinal cord. In spite of the tight apposition of the mass to the spinal cord (Figure 1A), as previously described,[7,17] an evident separation between the spinal cord and the adjacent mass could be observed (Figures 2B and 4B). There was typically little inflammatory infiltrate. The collection of cells constituting the mass surrounding the catheter track was largely composed of fibroblasts (Figure 4). Using Picro-Sirius and birefringence imaging, thick (yellow-orange birefringence) and thin (green birefringence) collagen fibers, indicative of Types 1 and 3 collagen, respectively, could be readily observed. Accordingly, Figures 4D and E show that staining was consistent with the matrix being largely Type 1 collagen fibrils. The results of the Herovici stain revealed ongoing collagen formation as evidenced by immature collagen adjacent to the catheter and mature collagen in the outer layers (Figure 3F). As previously described, the cellular composition of the mass in these models, aside from fibroblasts, consisted of macrophages and very few lymphocytes.[7,11,15]

Intrathecal mass size and neurological sequelae

Yaksh SDC Figure 2 presents a regression showing the number of adverse events observed as a function of mass size that revealed no evident correlation. There was an evident absence of adverse events in animals receiving intrathecal saline.

Dye diffusion profile.

Using a two-dimensional diffusion chamber to assess the movement of blue dye from the catheter (Model 8709SC) for drug delivery in the dog connected to a SynchroMed II pump, we examined the distribution of dye at 10 min after the delivery of a fixed volume (3.3 μL) as a bolus at 1000 μL/hr (16.6 μL/min) or the delivery of the same volume (3.3 μL) over 10 min at the rate of 20μL/hr (0.33 μL/min). Figure 5 presents representative images taken at 10 min after continuous infusion (Figure 5A, left) or bolus delivery (Figure 5A, Right). For each figure an enlargement is presented with arrows showing location of the exit port. Three characteristics were made from the visual observations provided in Figure 5A, confirmed by the densitometry assessments (5B and 5C): i) with bolus delivery, dye exited in one of the first ports (proximal to the pump). In contrast, with bolus delivery, dye exited bilaterally from the first and second ports. ii) Following continuous infusion, dye displayed an intense local distribution immediately adjacent to the exit port. and then a rapid taper in density. In contrast, after bolus delivery, dye showed peak densities bilaterally at a distance of several mm from the wall of the catheter. These observations are recapitulated in the dye density measurements. As shown in Figure 3B, peak density was observed asymmetrically immediately adjacent to the catheter while after bolus delivery (Figure 3C) the dye distribution was symmetrical with peak densities several mm adjacent to the catheter wall. As indicated in the Figure 5B and C, group peak densities with infusion were approximately twice that of the bolus delivery. iii) As shown in Figure 5 B and C, consistent with the reduced peak distribution the bilateral spread was greater bolus than infusion. In Figure 3D, the table presents the mean ± SE of the individual peak densities and bilateral lateral spread observed in each run for continuous and bolus delivery. As indicated, similar results were

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noted in this second analysis in terms of peak density (Infusion > bolus) and bilateral spread (Bolus > Infusion). Representative videos showing the 10 min. time course from which these results with bolus and continuous infusion were obtained are presented in video files (see Supplemental Digital Content video file 1 and file 2).

DISCUSSION:

The Intrathecal Mass.

The appearance of a space-occupying mass as a consequence of continuous intrathecal infusion of morphine has been shown [8] by histochemistry and/or MRI imaging in the guinea pig,[11] dog,[7,14] sheep[10] and human.[5,6] The present and previous work showed that the morphine-induced mass originates from the dura-arachnoid and not from the adjacent pia or spinal cord.[7] While often referred to as a collection of inflammatory cells and termed an “inflammatory” mass, the bulk of the mass is composed of fibroblasts and collagen.[11,18] In those studies, and in the present work, a progressive expansion was observed with less mature (newly secreted) collagen near the catheter and with more mature collagen present in the outer margin of the mass.

Role of meningeal mast cells

The mass is not a simple reaction to the catheter/infusion itself,[7,17] infection[7,19] or the formulation[20] but is produced by several (morphine, hydromorphone, methadone) but not all (fentanyl, alfentanil) opioids.[7–9,18] This differential effect of the several opioids (e.g. anilinopiperadines vs. morphine) is consistent with the failure of opioid antagonism to block mass formation.[15] As mass formation by morphine is reduced by mast cell stabilizers such as cromolyn sodium,[15] we believe that the mass is produced by degranulation of meningeal mast cells releasing agents that stimulate fibroblastic activity and collagen deposition [21–23] or by a direct effect upon fibroblast proliferation.[24] We note that all animals received diphenhydramine prophylactically to minimize scratching which can sufficiently severe as to terminate a study. Thus, while there are data showing that histamine (acting through an H1 receptors) can increase fibroblast proliferation ([25] but see [26]) and migration ([27]), the results observed with concurrent diphenhydramine dosing of all groups revealed that those animals receiving a continuous delivery displayed greater mass formation than those in the bolus paradigms. In canine studies, fentanyl and alfentanil, unlike morphine, do not degranulate mast cells[28] or produce IT masses.[9] Current work has emphasized the role of a family of Mas-Related G-Protein Coupled Receptors (MRGP-r) in mediating opioid-receptor-independent degranulation of mast cells by morphine.[29,30] In recent studies in the guinea pig, we found that two mu agonists, DMT-DALDA and PZM21, that are not activators of MRGP-r produce little or no mast cell degranulation, do not activate fibroblasts and when infused intrathecally do not produce intrathecal masses. Of note, it was recently reported that a spinal mass resected from a patient receiving 50 mg/mL morphine displayed particulates of morphine, in a loculated fluid -filled sac at the catheter tip, suggesting that at concentrations close to solubility in a buffered water environment with restricted diffusion access, particulate formation may occur and may contribute to the local reaction.[31]

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Bolus vs. Intrathecal Drug Delivery in Intrathecal Mass Formation.

An important variable in the formation of intrathecal masses is the role of opioid concentration. Previous work and that described here has shown that the development of an intrathecal masses in the canine model occurs with a continuous fixed rate infusion of morphine at doses of around 12 mg/day.[7,8,14] Systematic studies have shown that the effect is dependent upon local morphine infusate concentration, as opposed to total daily dose.[8] In humans, we noted that during the 10–15 years after the first intrathecal morphine infusion,[3] intrathecal masses were not reported and, at that time, the highest concentrations employed were typically in the range of 10 mg/mL.[13] The first clinical report of a spinal mass with continuous morphine infusion was observed in 1991 in a patient receiving 20 mg/mL morphine[4] and that concentration parameter has persisted in the numerous case reports since then [see [5,6,32]]. Of note, an early dog study revealed no untoward pathology to repeated intrathecal boluses of 10 mg/mL of morphine.[16] These results suggested that the use of a rapid bolus delivery might lead to a decreased concentration near the catheter tip and reduce the risk of mass formation. Preclinical work indeed demonstrated greater infusate distribution (CSF and spinal cord tissue) when higher infusion rates were employed.[33] In the present study, we observed that the rank order of cross-sectional mass area was greatest in the animals with continuous delivery, while daily doses delivered as a bolus at 1 mL/h (the highest rate possible with SynchroMed II) in divided volumes showed highly significant reductions in mass cross-sectional areas. Further, using an unstirred two-dimensional diffusion cell, we showed that infusion at 20 μL/hr (0.3 μL/min) through the human catheter, as employed in these canine studies resulted in dye movement through the most (to the pump) of the 6 exit ports (see Figure 5). This resulted in a steep concentration profile adjacent to the catheter lumen. In contrast, delivery of the same fixed volume as a bolus at 1000 μL/h (16.6 μL/min) resulted in bilateral dye movement from other ports, significantly reduced peak dye concentration adjacent to the catheter tip and a corresponding increase in lateral distribution of the dye as compared to the same volume delivered as a slow infusion. These results are thus jointly consistent with the hypothesis that delivery of the infusate dose as a series of boluses results in greater distribution and lower peak concentrations adjacent to the catheter.

Bolus vs. Intrathecal Drug Delivery in Intrathecal Mass Formation.

An important concern is the effects of multiple boluses on the analgesic profile. Such assessments were not performed in these animals. However, it is currently appreciated that the therapeutic effects of intrathecal infusion may be improved in some patients if the total daily dose/volume is delivered as a series of rapidly infused boluses in contrast to a continuous slow rate, e.g. 20 μl/h or less as often employed in current intrathecal indications for chronic pain[34,35] and severe spasticity.[36–38]

Theoretical Implications of Data

These results reflect upon the practical significance of the issues raised by Bernards and others that the spinal CSF represents a poorly mixed fluid with the properties of a “backwater bayou as opposed to a river” and further emphasize the importance of developing optimal delivery protocols[39] and systems that lead to increased local infusate

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movement by increasing exit velocities of the infusate in low volume delivery scenarios which is usually the case with implantable drug pumps with limited volume reservoirs that must be refilled on a regular basis.[20]

The present studies employed several divided dose protocols. The x1, x2 and x4 protocols all displayed significant reductions in spinal-mass-inducing activity as compared to the x8 boluses and yet greater differences vs. the continuous delivery treatment group. Whether the x8 mass incidence is an anomaly of the data set or represents an effect reflecting an ongoing exposure cannot be determined from this study. Previous work has shown that intrathecal morphine as a bolus typically displays a half-life in human and animal CSF of approximately 2–3 h,[16,40,41] a finding consistent with the observation of a dose-dependent duration of analgesic action of 4–12 h in the dog and human. Accordingly, the 6-h interval between boluses deliveries in the x4 group for morphine appears to be a rational choice. Drugs showing a shorter or longer half-life would reasonably allow differences in the appropriate inter-bolus interval.

The present studies employed a standard large multiport catheter that is approved for long-term intrathecal infusion in humans. As shown in the simple bench studies, low rate (0.33 μL/min) continuous infusion of the dye essentially exited the first port of the catheter (e.g. proximal to the pump). At the bolus rate (16.7 μL/min), the same volume delivered showed i) a symmetrical bilateral distribution, ii) movement of dye from the second set of ports and iii) correspondingly significantly reduced peak concentrations at the first set of ports. These parameters are a function of the exit orifice diameter. Predictably, smaller exit orifices will increase the exit velocity for a given flow rate and enhance distribution. We suggest that a possible development in future catheters will employ catheter with substantially smaller outlet portals, resulting in higher exit velocities for a given volume delivered over a minimal length of time and thus more equitable egress of fluid between the first and last portal.[20] We believe that these studies, though associated with morphine, have broad relevance to the neuraxial delivery of other agents where enhanced local redistribution may enhance safety and efficacy.[42–44]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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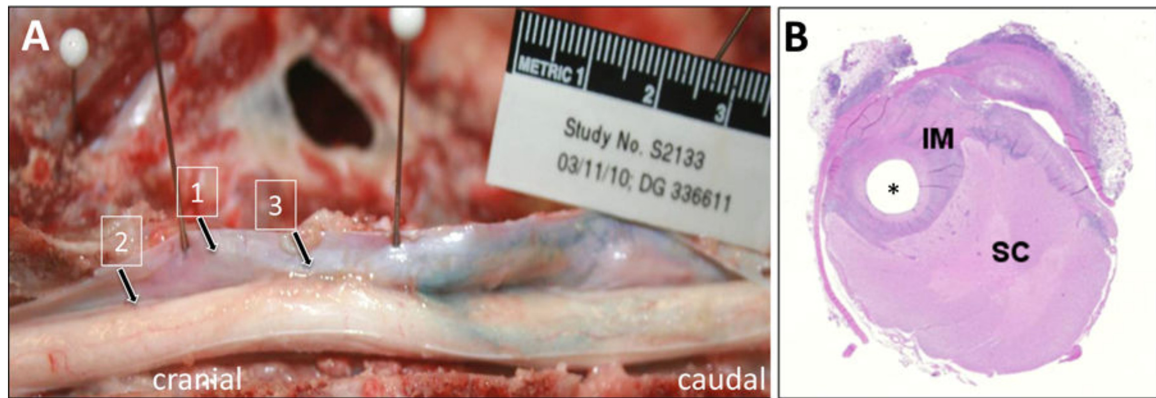

Figure 1.

1A. Representative image of gross pathology of spinal cord after 28 days of continuous intrathecal infusion of morphine sulfate (Infumorph® 500, 25 mg/mL, 12 mg/day at 0.48 mL/day). Arrow 1:retracted dura. Arrow 2: intrathecal catheter passing along dorsolateral aspect of spinal cord; Arrow 3: opaque mass approximately 2–3 cm in length with evident adhesion to adjacent meninges. Blue color caudal to mass shows presence of methylene blue injected at necropsy.

1B. presents the associated microscopic pathology with hematolylin-eosin staining of this spinal cord section, revealing a large space-occupying intrathecal mass (IM) that produces a prominent distortion of the spinal cord parenchyma(SC). The asterisk denotes the space occupied by the catheter.

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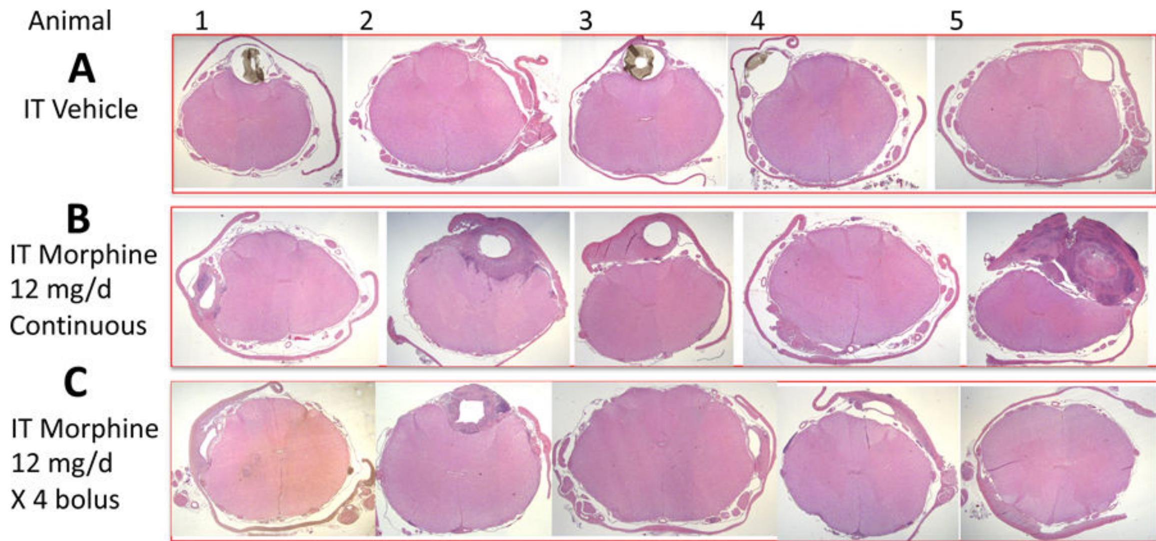

Figure 2.

Histology of lumbar sections near the catheter tip (stained with hematoxylin-eosin) after: A) intrathecal saline (0.48 mL/day, N = 5) and B) the continuous intrathecal infusion of morphine (25 mg/mL/0.48 mL/day at 20 μL/h, N = 5) and C) the intrathecal infusion of morphine (25 mg/mL/0.48 mL/day) at 1000 μL/h delivered in 4 equally divided bolus infusion episodes at 6-h intervals (N = 5).

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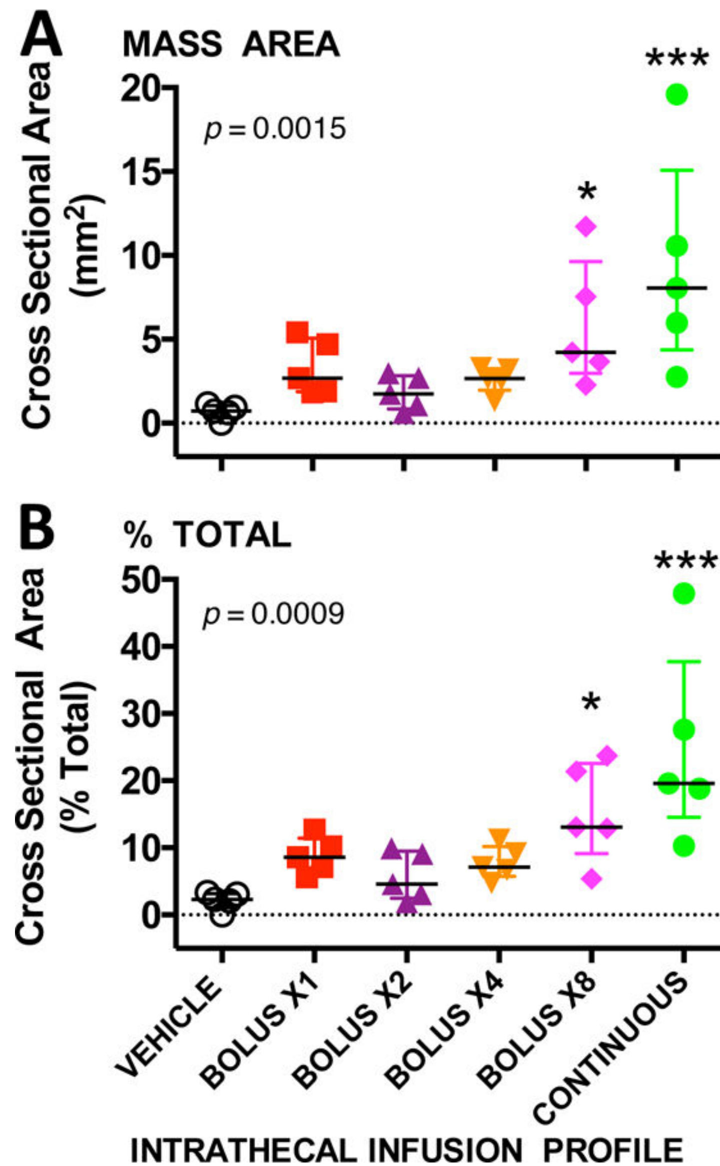

Figure 3.

Scatter plots for each animal in the 6 treatment groups, showing: A) cross-sectional area (mm^2) and B) the percent of the cross-sectional area of the mass vs. the total cross-sectional area of the spinal cord in that section for animals receiving 0.48 mL/da of 25 mg/mL morphine as a continuous infusion or as a single bolus, or in equidived doses equally divided doses at intervals of 12 hrs (x2); 6 hrs (x4) or 3 hrs (x8). A Kruskal Wallace analysis showed significant main effects. Post hoc comparison to the saline (vehicle control) group using Dunn's test for multiple comparison: * $p<0.05$; ***: $p<0.001$.

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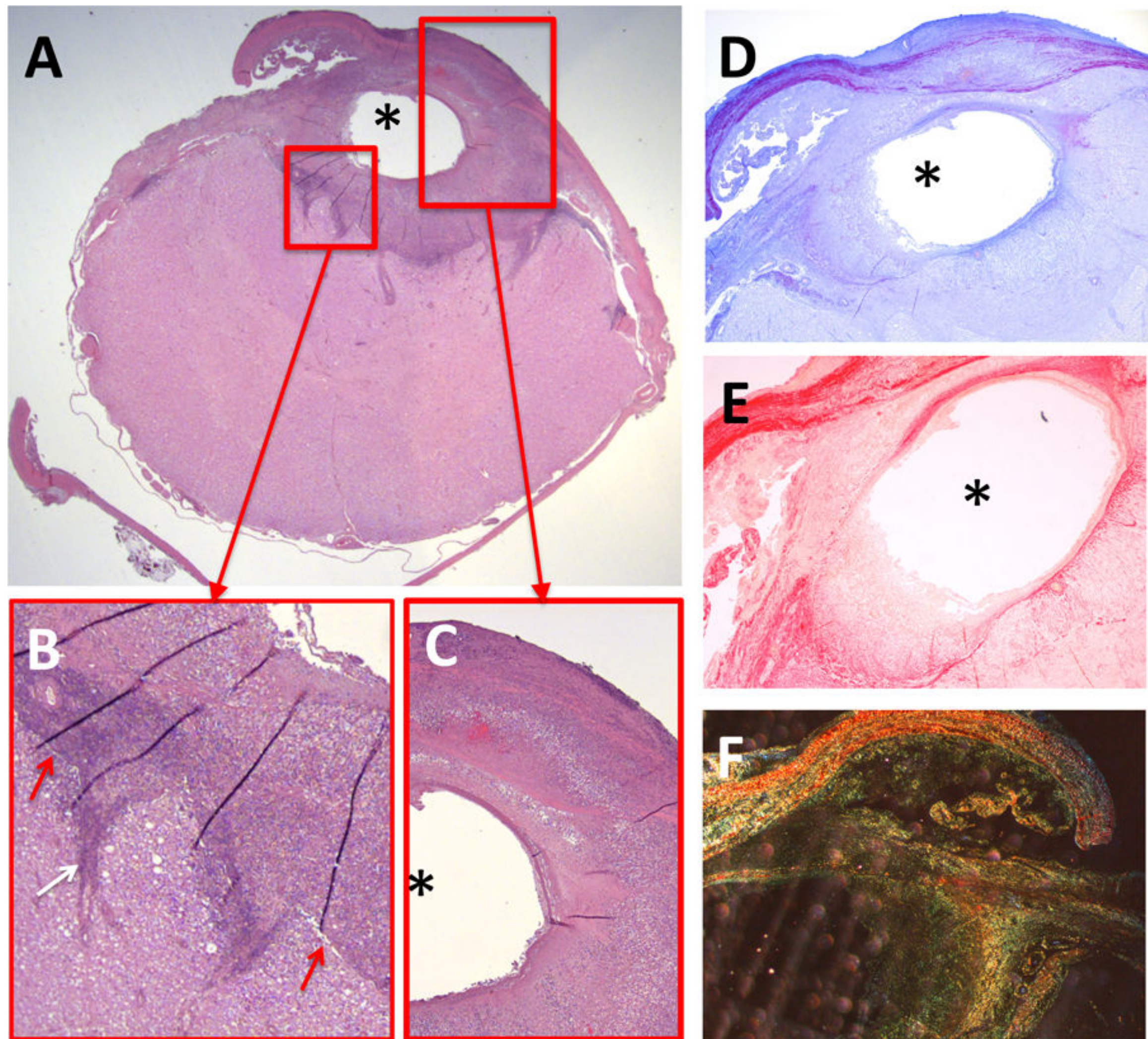

Figure 4.

Histochemistry of lumbar spinal sections (taken adjacent to section shown in Figure 1A. from one animal receiving IT infusion of morphine (12 mg/0.48 ml/day) for 28 days (4A was shown previously in Figure 1). 4B and 4C show enlargements of the area outlined in 4A. Red arrows indicate demarcation between the IT mass and the spinal cord. The white arrow shows the presence of fibroblasts and collagen in the Virchow Robbins space. 4D. Shows the Herovichi-stained section of adjacent spinal tissue where young collagen is indicated in blue and more mature collagen in red. 4E. shows Picro-Sirius staining in normal white light microscopy where collagen appears red. 4F. displays the image in polarized light where type 1 collagen is indicated by yellow-orange birefringence and type 3 collagen by green refringence. *indicates catheter lumen.

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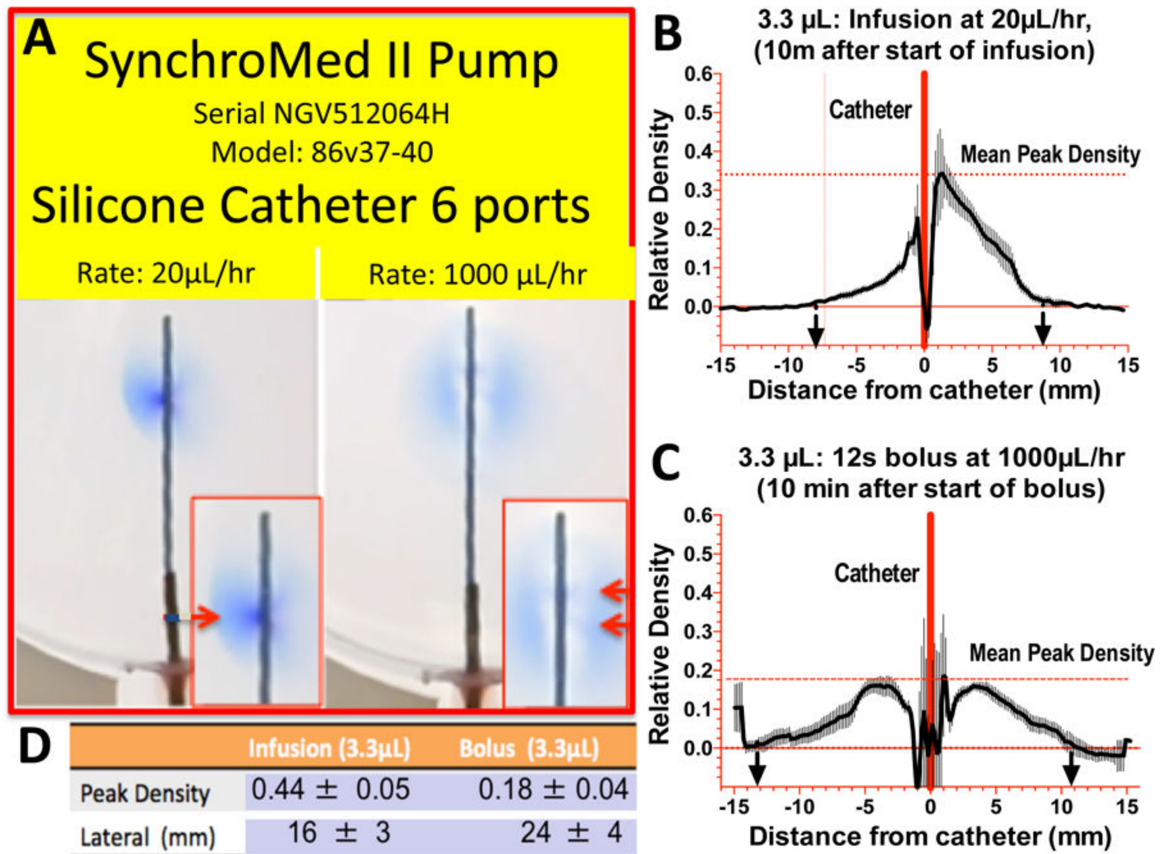

Figure 5.

5A. Photograph of the two-dimensional diffusion chamber assessing movement of blue dye from the same type of catheter (Model 8709SC) employed in the canine study connected to a SynchroMed II pump. The most proximal (to the pump) pair of ports are located at 180° from each other and the catheter is arranged so that the axis of these first two ports are parallel with bottom of the chamber. Left: Image taken at 10 min after the start of a continuous infusion of 2% methylene blue dye at 20 µL/hour delivering 3.3 µL. Right: 10 min following the bolus delivery of 3.3 µL at 1000 µL/h.

5B. Mean ± SEM (N = 5 replications) densitometry measurements (arbitrary units) across a line perpendicular to the catheter at the point of dye exit from the first port proximal to the pump as shown in 5A left and bolus as shown in 5A right.