Time Course and Role of Morphine Dose and Concentration in Intrathecal Granuloma Formation in Dogs

A Combined Magnetic Resonance Imaging and Histopathology Investigation


Background: Intrathecal morphine infusion leads to intrathecal granulomas. In dogs, the authors examined time course of granuloma formation and the role of concentration in granuloma development.

Methods: Dogs were prepared with lumbar intrathecal catheters and vest-mounted pumps. To define the time course of granuloma formation, serial magnetic resonance imaging was performed in animals receiving 10 or 31 days of morphine infusion (12.5 mg/ml at 40 µl/h). At these times, morphine was removed from the infusate, and further magnetic resonance images were acquired over 14–35 additional days. To assess dose versus concentration, dogs received 28-day infusions of vehicle, 12 mg morphine/day as 12.5 mg/ml at 40 µl/h, or 1.5 mg/ml at 334 µl/h (12 mg/day) for 28 days. Additional dogs received 3 mg/day as 12.5 mg/ml at 10 µl/h.

Results: Serial magnetic resonance images in dogs receiving morphine (12.5 mg/ml at 40 µl/h) revealed pericatheter-enhancing tissues as early as 3 days with a prominent signal by 10 days. Removal of morphine reduced the mass volume within 7 days. At a fixed infusion rate, the incidence of granuloma formation with the continuous intrathecal infusion of morphine ranged from 0 in vehicle-treated dogs to 100% in dogs treated with 12.5 mg/ml at 40 µl/h (12 mg/day). Infusion of 12 mg/day at 1.5 mg/ml (334 µl/h) resulted in granuloma in one of four animals. The authors found that infusion of morphine in different concentrations at a fixed rate resulted in a dose-dependent increase in concentration, with the granuloma-producing, dose-yielding lumbar cerebrospinal fluid morphine concentrations around 40 µg/ml.

Conclusions: Serial magnetic resonance imaging showed a rapid formation and regression of the masses initiated by intrathecal morphine infusion. These masses are dependent on local concentration.

AFTER initial characterization of the analgesic actions of intrathecal opiates in animals,1 spinal delivery was widely initiated in humans for the management of acute pain. Implementation of chronic indwelling intrathecal catheters with implantable pumps permitted the routine continuous delivery of spinal opiate analgesics.2 Starting in 1991,3 clinical case reports began to be published that described patients receiving chronic morphine infusion who presented with a motor or sensory dysfunction secondary to an extraparenchymal mass localized at the catheter tip producing compression of the spinal cord.4–10 In humans receiving chronic intrathecal opiate infusions where masses were surgically resected, the typical histology emphasized the presence of macrophages, neutrophils, and monocytes, with a necrotic center and no evidence of an infectious process.6,11 Studies initiated in the chronic intrathecally catheterized canine model confirmed that intrathecal morphine sulfate produced a local compressing mass when infused over 28 days.12 Comparable effects have been observed in sheep.13 These inflammatory masses have been termed granulomas in the literature, in part because they contained signs of both acute and chronic inflammatory processes. These masses are in fact an accumulation of granulation tissue, not meeting the classic definition of a granuloma, which requires the presence of giant cells. Therefore, although the term granuloma has become common for these inflammatory masses, it is important to clarify that these masses do not meet the formal histopathologic classification of a granuloma.

Several issues related to the formation of intrathecal granulomas are of both a basic science and clinical interest. First, the phenomena seem to be relatively frequent. Although retrospective reviews suggest an overall incidence of approximately 0.1% in a population of approximately 13,000 intrathecal pump patients, this is based on the alerting presence of neurologic signs14 and may underestimate the true incidence. Therefore, in one case series, seven patients were imaged, and three of those patients displayed evident masses.7 Second, the time required for human granuloma development or its reversibility is not certain. Third, it is unclear whether total daily dose or infusate concentrations is of greater importance in mass formation. Characteristically, patients developing granulomas received high concentrations of morphine (e.g., in the range of 25–50 mg/ml where reported). Historically, since the mid-1980s, daily intrathecal doses in humans have remained comparable (around 10 mg/day), but the onset of reported granulo-
mas in 1991 coincided with the beginning of a trend to use much higher morphine concentrations (e.g., 10 vs. 25–50 mg/ml). This raises the possibility that local concentration, as opposed to total daily dose, may be the most important factor in determining granuloma formation.

The current studies seek to characterize the role of concentration and dose of morphine in the intrathecal infusion in chronically prepared dogs and to define the time course of onset and regression of the intrathecal granuloma using both magnetic resonance imaging (MRI) and histochemical analysis.

### Materials and Methods

All studies described here were accomplished under method protocols approved by the Institutional Animal Care and Use Committee of the University of California, San Diego.

**Animals**

Beagle dogs (Marshall Farms, North Rose, NY), aged 12–16 months and weighing approximately 12–15 kg, were individually housed in runs with wood shavings and given *ad libitum* access to food and water with lighting set on a daily 12-h light–dark cycle. Kennel temperatures were maintained within the range of 16.5°–27.8°C. Animals were adapted to testing protocols for a minimum of 10 days before surgery. A nylon vest (Alice King Chatham Medical Arts, Hawthorne, California, or Lomir, Montreal, Quebec, Canada) was placed on each dog approximately 48 h before scheduled intrathecal catheter placement surgery for acclimation.

**Surgical Preparation of Animals**

For chronic intrathecal infusion, dogs were prepared with chronic intrathecal catheters. Surgical placement of the intrathecal catheter was accomplished approximately 72 h before initial dosing. Prophylactic antibiotic (sulfamethoxazole:trimethoprim [200:40 mg tablet], oral, twice daily) was given 48 h before surgery and for 48 h after surgery. The dogs received atropine (0.04 mg/kg intramuscular) before sedation with xylazine (1.5 mg/kg intramuscular). Anesthesia was induced with 4–5% isoflurane in 50:50 oxygen and nitrous oxide. Dogs were intubated, and anesthesia was maintained under spontaneous ventilation with 1.0–2.0% isoflurane. Intraoperatively, animals were continuously monitored for oxygen saturation, inspired, and end-tidal values of isoflurane, carbon dioxide, nitrous oxide, and oxygen and heart and respiratory rates. Surgical areas were shaved and prepared with chlorhexidine. Using sterile technique, a midline skin incision was made at the dorsal neck, and the dura overlying the cisterna magna was exposed by dissection. Through a small dural incision (1–2 mm), the PE-10 intrathecal catheter (fabricated of polyethylene tubing and sterilized by E-beam irradiation) was inserted and passed caudally at a distance of approximately 40 cm to a level corresponding to the L2–L3 vertebral segment. A single dexamethasone sodium phosphate dose (0.25 mg/kg intramuscular) was administered just after catheter placement to lessen any acute inflammatory reaction induced by catheter placement and to provide adjunctive postsurgical analgesia. The external catheter was then tunneled subcutaneously to exit at the left scapular region. The incision was closed in layers with a 3-0 suture. Upon closure, the isoflurane–nitrous oxide was discontinued, and the animal was observed during recovery. Butorphanol tartrate (0.04 mg/kg intramuscular) was administered upon recovery and as necessary to relieve postoperative discomfort. For assessment of cerebrospinal fluid (CSF) concentrations in dogs receiving continuous infusions, separate animals were prepared as described above, and a second catheter (polyethylene, PE-50) was placed in addition to the infusion catheter for purposes of lumbar CSF sampling. The sampling catheter was passed such that the tip was approximately 1–2 cm rostral to the infusion site and externalized. After anesthetic recovery, a nylon vest was placed on the animal, and an infusion pump (PANOMAT C-10; Disetronic Medical Systems, St. Paul, MN; or equivalent) was secured in the vest pocket where it was connected to the externalized end of the PE-10 intrathecal catheter. Animals received saline, 40 μl/h, for approximately 3–5 days after surgery.

**Clinical Observations**

Daily observations included temperature measured using an ear thermometer and general behavior. Specific behavioral indices of arousal (−3 to +3), muscle tone (−3 to +3), and coordination (0 to 3) were assessed. Normal function received a score of 0, whereas scores of 1, 2, or 3 represented mild, moderate, or severe abnormalities, respectively. Cumulative motor function scores were determined by summing the absolute value of the muscle tone and coordination scores. A 28-day cumulative score for each behavioral parameter (arousal, muscle tone, coordination) was obtained by summing absolute values of daily scores. These indices have been previously validated.

**Necropsy**

For euthanasia, dogs were deeply sedated with acepromazine (10 mg intramuscular) and anesthetized with an intravenous dose of sodium pentobarbital (35 mg/kg or dose to effect). After a satisfactory level of anesthesia was achieved, cisternal CSF and plasma samples were obtained by percutaneous puncture. After CSF
Sampling, the animals were exsanguinated by perfusion with saline followed by 10% neutral-buffered formalin (each approximately 4 l) delivered by roller pump at approximately 100 mmHg. The spinal cord and lower brainstem were exposed by dorsal laminectomy of the vertebral column. Methylene blue dye was injected through the catheter to visualize the position of the intrathecal catheter tip. The condition of the spinal cord was noted, and in selected animals, photographs were taken of the spinal cord and catheter tip in situ. The spinal cord was resected at specific regions and, taking care to keep the dura intact, placed in fixative (10% neutral-buffered formalin) for histopathologic analysis. Spinal cord sections were embedded in paraffin, sectioned at approximately 8 µm, and stained with hematoxylin and eosin.

**Drug Infusates**

Drug formulations used in this current series of studies were prepared from chemically pure reagents obtained from commercial sources at the highest available grade of purity. Morphine solutions were by dilution of Inuf orm (25 mg/ml; Elkins-Sinn, Inc., Cherry Hill, NJ). Drugs were prepared from the powder after aseptic precautions. The routine vehicle was sterile, preservative-free US Pharmacopeial saline (0.9%) or water for injection. For high concentrations, solutions were prepared in sterile, pyrogen-free, 10-ml vials for single use only and stored at 4°–22°C until used.

**Drug Analyses**

Analyses of blood and CSF morphine/metabolite concentrations were performed by the University of California, San Diego Anesthesiology Research Assay Laboratory following a previously published protocol. Specifically, morphine and the 3-O- and 6-O-ester glucuronides were quantified after extraction from biologic matrices by mixed-mode solid phase cartridge chromatography. After desiccation under purified nitrogen, morphine and glucuronides were separated by capillary matrices by mixed-mode solid phase cartridge chromatography. After desiccation under purified nitrogen, morphine and the 3-0- and 6-0-ester glucuronides were separated by high-performance liquid chromatography and quantified by comparison of ultraviolet absorbance with that of external standards, after correction for recovery error by internal standardization. This method has a limit of detection of 0.1 ng/ml with a sample minimum volume of 100 µl.

**Study Protocols**

The following studies were undertaken in this report:

**Time Course of Granuloma Formation.** Dogs were prepared with lumbar intrathecal catheters, and 3–5 days later (day 0), intrathecal morphine infusions (12.5 mg/ml at 40 µl/h) were initiated. This resulted in 12 mg morphine sulfate/day delivered in a volume of 0.96 ml. MRI scans were obtained after morphine infusion (day −3), 3 days after initiation of morphine infusions (day 3), and then at approximately 7-day intervals. Dogs, n = 3/group, were assigned to receive one of the following treatments:

**Group 1:** Dogs were continuously infused with morphine until approximately day 10. At that time, the animals were necropsied. Ten days was chosen because preliminary MRI studies determined that all dogs showed evident mass formation at this time point.

**Group 2:** Dogs were continuously infused with morphine until approximately day 10. At that time, the infusate was changed to saline vehicle, which was diluted to 2.5 mg/ml with D5W, enabling an infusion rate of 0.1 ml/kg·min (60 ml/h for a 10-kg dog). After anesthesia induction, dogs were intubated and connected to MRI-compatible pulse oximeter/respiratory gas sensors that were connected to a monitor (In Vivo, Orlando, FL) for continuous assessment of oxygen saturation, end-tidal carbon dioxide, respiratory rate, and heart rate throughout the duration of anesthesia. After completion of the scan, the propofol infusion was terminated, and dogs were extubated upon arousal. MRI scans were obtained before and after intravenous bolus of a gadolinium chelate of diethylenetriamine pentaacetic acid bismethoxyethylamide (OptiMARK; Mallinckrodt, Hazelwood, MO). Intrathecal gadolinium, 1 ml Magnevist, 1.25 µm (gadolinium diethylenetriamine pentaacetic acid N-methylglucamine; Berlex, Montville, NJ), was also injected in some animals after the postintra-venous contrast scans. High-resolution MRI images were acquired using a Siemens Symphony scanner (Siemens Medical Solutions USA, Inc., Malvern, PA) at 1.5-T equipped with high-performance gradients. For this study, we used the phased-array coils embedded in the scan table and positioned the dogs in the supine position. After localizer scans obtained in the sagittal plane, high-resolution T1- and T2-weighted images were acquired to cover the lower thoracic and lumbar vertebrae. High-resolution, fat-suppressed T1-weighted images were then acquired 1 min after gadolinium administration in the transverse plane (252-s acquisition time), followed by the sagittal plane (78-s acquisition time). These two series were repeated when intrathecal gadolinium was given.

**In Vivo Imaging**

For imaging, dogs were premedicated with 0.4 mg/kg atropine sulfate intramuscular. Anesthesia was induced intravenously with 4 mg/kg propofol (1%, 10 mg/ml). For constant infusions, the propofol solution was diluted with saline followed by 10% neutral-buffered formalin (each approximately 4 l) delivered by roller pump at approximately 100 mmHg. The spinal cord and lower brainstem were exposed by dorsal laminectomy of the vertebral column. Methylene blue dye was injected through the catheter to visualize the position of the intrathecal catheter tip. The condition of the spinal cord was noted, and in selected animals, photographs were taken of the spinal cord and catheter tip in situ. The spinal cord was resected at specific regions and, taking care to keep the dura intact, placed in fixative (10% neutral-buffered formalin) for histopathologic analysis. Spinal cord sections were embedded in paraffin, sectioned at approximately 8 µm, and stained with hematoxylin and eosin.

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Drug formulations used in this current series of studies were prepared from chemically pure reagents obtained from commercial sources at the highest available grade of purity. Morphine solutions were by dilution of Inuf orm (25 mg/ml; Elkins-Sinn, Inc., Cherry Hill, NJ). Drugs were prepared from the powder after aseptic precautions. The routine vehicle was sterile, preservative-free US Pharmacopeial saline (0.9%) or water for injection. For high concentrations, solutions were prepared in sterile water and osmolarity and were brought to approximately 300 mOsm with saline. All solutions were prepared in sterile water and osmolarity and were brought to approximately 300 mOsm with saline. For high concentrations, solutions were pre-
continuously infused until approximately day 45, at which time the animals were necropsied.

Group 3: Dogs were continuously infused with morphine until approximately day 30–31. At that time, the infusate was changed to saline, which was continuously infused until approximately day 45, at which time the animals were killed and underwent necropsy.

Role of Morphine Dose and Concentration. To assess the role of intrathecal morphine dose versus concentration, dogs with chronic intrathecal dosing catheters were also prepared with chronic lumbar intrathecal sampling catheters and assigned to one of the following treatment groups outlined in table 1. The model used here is identical to that reported previously with intrathecal morphine displaying CSF morphine levels obtaining steady state at this time point. Accordingly, for analysis purposes, the results from animals in several groups reported in that study are included in these studies as indicated in table 1.

Intrathecal Morphine, Morphine-3-glucuronide, and Morphine-6-glucuronide Metabolite Concentration. To determine the lumbar CSF concentrations that were associated with granuloma formation in the several infusion paradigms, we prepared additional dogs with a chronic lumbar infusion and a sampling catheter that lay 1–2 cm from the infusion tip. After a minimum of 24 h of continuous lumbar drug infusion, venous blood samples and lumbar CSF samples were obtained from the lumbar intrathecal sampling catheter and were frozen at −20°C until assayed for morphine and the 3 and 6 glucuronides.

Results

Intrathecal Mass Formation

MRI and Histology. The intrathecal infusion of morphine (12.5 mg/ml at 40 μl/h) reliably produced a granuloma proximal to the catheter tip. At necropsy, the mass typically presented as a significant discoloration of the dura with adherent epidural fat, and a grossly evident spinal cord deformation that extended approximately four to five spinal segments with the catheter tip in the middle. Figure 1 displays an axial and sagittal MRI correlated with histology obtained within 24 h of the MRI images in a dog that had received 10 days of intrathecal morphine. As evidenced in this T1-weighted, systemic gadolinium-enhanced scan, there was a clear depiction of a space-occupying mass at the corresponding level of the L2 catheter tip. The MRI and histology displayed a mass that was centered at L2 but extended approximately 2–3 cm rostrally and caudally.

Time Course of Mass Appearance. To define the time course of granuloma formation, we used serial MRIs. Dogs with intrathecal catheters were imaged before and at intervals after the initiation of intrathecal infusion of 12.5 mg/ml morphine at 40 μl/h. Figures 2 and 3 displayed a typical MRI study sequence for a short
(10 days) and extended (30–31 days) intrathecal morphine infusion. A modest mass was observed at intervals as short as 3 days, and this mass became substantial by 10 days. This profile of mass development was extremely reproducible, being observed in all six dogs that underwent this morphine infusion and were examined with serial MRI (table 2).

**Time Course of Mass Regression.** To define the reversibility of the granuloma formation, the animals described above were converted to saline infusion for an additional 30 days for those dogs whose morphine was stopped at day 10 or days 30–31. As indicated in figure 3 (10-day reversal) and figure 4 (31-day reversal), over the 14- to 30-day interval, all masses displayed a reduction in size. However, the older the mass, the less regression was noted. These MRI sequences are representative of the three dogs in each group (table 2).

**Correlation of MRI with Motor Function.** In previous work, in the intrathecal morphine infusion canine model we found evidence of hind limb spasticity, hyperreflexia (increased hind limb motor tone), and extreme sensitivity to light touch (hyperesthesia) in approximately half of the dogs at intervals as early as 10–14 days. Subsequent analysis of MRI gross necropsy and histopathology indicated that there was little correlation between the size of the intrathecal mass and the behavioral dysfunction shown by the animal. Therefore, although all animals with abnormal neurologic signs had significant granuloma-related cord compression, some dogs with an evident mass did not show any distinctive behavioral signs. However, in the current study, with the regression of the mass after termination of morphine infusion as visualized on MRI, behavioral signs, when present, typically showed improvement (table 2).
Table 2. Time Course of Appearance/Disappearance of Granuloma and Changes in Motor Tone with Intrathecal Infusion of Morphine (12 mg·ml⁻¹·day⁻¹) for 10 or 31 Days Followed by 14 Days of Saline (n = 3/Treatment)

<table>
<thead>
<tr>
<th>Time Course</th>
<th>Morphine infusion</th>
<th>Granuloma</th>
<th>Hind limb tone</th>
<th>Hyperesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day -3</td>
<td>No</td>
<td>0, 0, 0</td>
<td>0, 0, 0</td>
<td>0, 0, 0</td>
</tr>
<tr>
<td>Day 3</td>
<td>Yes</td>
<td>0, 0, 1</td>
<td>0, 2, 0</td>
<td>0, 0, 0</td>
</tr>
<tr>
<td>Day 10</td>
<td>Stop</td>
<td>2, 2, 2</td>
<td>0, 1, 0</td>
<td>0, 0, 0</td>
</tr>
<tr>
<td>Day 24</td>
<td>No</td>
<td>0, 1, 1</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Time Course</th>
<th>Morphine infusion</th>
<th>Granuloma</th>
<th>Hind limb tone</th>
<th>Hyperesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day -3</td>
<td>No</td>
<td>0, 0, 0</td>
<td>0, 0, 0</td>
<td>0, 0, 0</td>
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<tr>
<td>Day 3</td>
<td>Yes</td>
<td>0, 0, 0</td>
<td>0, 0, 0</td>
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<tr>
<td>Day 10</td>
<td>Yes</td>
<td>0, 0, 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Day 17</td>
<td>Stop</td>
<td>2, 2, 2</td>
<td>0</td>
<td>0</td>
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<td>Day 24</td>
<td>Yes</td>
<td>0, 0, 0</td>
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<td>Day 31</td>
<td>No</td>
<td>0, 0, 0</td>
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<td>Day 39</td>
<td>No</td>
<td>0, 0, 0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>Day 45</td>
<td>No</td>
<td>0, 0, 0</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

* Granuloma scoring: 0 = no detectable signs at or near the catheter tip; 1 = mass evident but produces little compression; 2 = mass produces some compression of one quadrant; 3 = mass produces compression of at least two quadrants.
† Hind limb tone: 0 = normal; 1 = mild paresis of one hind limb; 2 = significant motor tone, enhanced hind limb reflex; 3 = severe disabling rigidity of hind limb.
‡ Hyperesthesia: 0 = normal; 1 = mild agitation when hindquarters are brushed or stroked; 2 = significant agitation when hind limbs are brushed or stroked; 3 = severe agitation/vocalization when hind limbs are brushed or stroked.

Infuse Concentration and Dose in Granuloma Formation by Intrathecal Morphine

Morphine Dose–Response Curve: Effect of Infusion Rate/Concentration. At a fixed infusion rate, the incidence of granuloma formation with the continuous intrathecal infusion of morphine delivered at 40 µl/h ranged from 0 in vehicle-treated dogs to 100% in dogs treated with 12.5 mg/ml (table 3). To assess the role of dose versus concentration, dogs received the standard 12 mg morphine per day dose, but at a concentration of 1.5 mg/ml and a rate of 334 µl/h (8 ml/day). As indicated in figure 4, the 12.5 mg/ml at 40 µl/h dosing paradigm resulted in a typical, large, compressive mass in contrast to the lack of effect of the same dose at the lower concentration (1.5 mg/ml). As shown in table 3, one out of four of the animals receiving 12 mg/day at a low concentration formed masses, whereas 100% displayed mass formation at the high concentration. In saline animals, mass formation has consistently been 0%. In an additional study, 12.5 mg/ml delivered at 10 µl/h (3 mg/day) resulted in a lower incidence of granuloma than observed at lower concentrations for dogs in group 5 (table 1). In these instances, the mass sizes were significantly smaller, being only several millimeters in length and girth when observed at necropsy and at histology. This is in contrast to a mass in animals receiving 12.5 mg/ml at 40 µl/h that routinely displayed a length up to 5 cm and occupied more than half of the radial volume of the spinal canal.

CSF Concentrations Related to Granuloma Development. Dogs were infused with the infusate morphine formulation listed below for a minimum of 24 h before CSF sampling. In preliminary studies, we compared concentrations of morphine and glucuronide metabolites at 24 and 48 h in the same animal and found no evident differences. This finding is consistent with previous work in this sampling model with intrathecal drugs infused continuously.16 The results of these studies are summarized in table 4.

As indicated in this model over the range of infusion concentrations 1.5–12.5 mg/ml delivered at a fixed rate of 40 µl/h, lumbar CSF concentrations ranged from 3 to 40 µg/ml. The conjugated metabolite concentrations were low (ng/ml range) and variable. The approximate ratio of CSF metabolites (M3G:M6G) to parent compound (M) after 12.5 mg/ml morphine was 0.003:0.001:1 (M3G:M6G:M).

With regard to the effects of infusate concentration,
the lumbar CSF morphine concentrations observed with the low concentration and high rate of infusion were unexpectedly identical to those observed with the higher concentration and low rate of infusion. Conversely, the high concentration infused lower lumbar CSF morphine concentrations. These data suggested that lumbar CSF concentrations were roughly proportional to total dose infused in the model when a fixed rate was used.

**Discussion**

**Intrathecal Morphine and Granuloma Formation**

In the dogs receiving intrathecal infusions of morphine, there is an evident dependency of the incidence of granuloma formation on infusate dose/concentration over a range of 1.5–12 mg · ml⁻¹ · day⁻¹, with the highest dose resulting in 100% incidence. In all cases, these granulomas were noted to arise from the dura arachnoid layers. These observations are similar to previous reports in dogs and sheep.¹²,¹³ The confound resulting from increasing dose with concentration led us to undertake a study comparing 12 mg/day delivered in a concentration of 12.5 mg/ml at 40 µl/h with 12 mg/day delivered in a concentration of 1.5 mg/ml at 334 µl/h. Here, the same daily dose delivered at a high concentration, but not a low concentration, resulted in reliable granuloma formation. It is not likely that simple dilution of potential inflammatory mediators due to the high infusate flow rate was responsible for this effect in the 1.5-mg/ml group because a previous study found a similar rate of mass formation with a 40-µl/h infusion. This observation is consistent with the hypothesis that infusate concentration is an important covariate. We sought to extend this observation by considering the granuloma-producing effects of a low daily dose (3 mg) delivered at the high concentration (12.5 mg/ml) but at a low rate (10 µl/h). Granulomas were present in this group, but the size of the masses were considerably smaller than in other groups, correlating with a smaller area of intrathecal tissue exposed to a local high concentration of morphine.

Using MRI, we were able to definitively correlate the histopathology with the MRI signal. Moreover, the serial MRI with intravenous gadolinium enhancement served to demonstrate the ability to visualize mass formation, the time course of its evolution, and the reversibility of the mass after termination of opiate infusion. Therefore, mass onset at an effect concentration was observed to occur by 10 days. Importantly, mass size did seem to reach a maximum size within the 30 days of infusion, and reversal of the mass was evident within 14 days after termination of infusion, although the degree of reversal was less in animals with longer established masses. Elements of these observations will be further considered below.

**Steady State Lumbar CSF Morphine Levels and Granuloma Formation**

To further consider the hypothesis of the role played by concentration in granuloma formation, we examined the steady state lumbar CSF morphine concentrations associated with the several infusion paradigms. We assume that in the face of continuous infusion, the concentration precisely at the tip must correspond to the infusate concentration (e.g., 1.5–12.5 mg/ml). The sampling catheters were prepared to be approximately 2–3 cm proximal to the tip. This distance corresponded to the typical rostral extent of the granuloma resulting from the 12.5 mg/ml at 40 µl/h infusion with the lumbar

**Table 3. Incidence in Dogs of Granulomas Defined by Magnetic Resonance Imaging or Necropsy with up to 28 Days of Intrathecal Infusion**

<table>
<thead>
<tr>
<th>Total daily dose, mg</th>
<th>12 mg/ml, 40 µl/h</th>
<th>6 mg/ml, 40 µl/h</th>
<th>1.5 mg/ml, 40 µl/h</th>
<th>1.5 mg/ml, 333 µl/h</th>
<th>12.5 mg/ml, 10 µl/h</th>
<th>Vehicle</th>
</tr>
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<tbody>
<tr>
<td>Incidence of granuloma</td>
<td>9/9</td>
<td>3/4</td>
<td>1/6</td>
<td>1/4</td>
<td>3/5</td>
<td>0/6</td>
</tr>
</tbody>
</table>

**Table 4. Lumbar CSF Concentrations of Morphine and Metabolites after 48 h of Lumbar Intrathecal Infusion**

<table>
<thead>
<tr>
<th>Infusate/Infusion Rate</th>
<th>12 mg/ml, 40 µl/h</th>
<th>6 mg/ml, 40 µl/h</th>
<th>1.5 mg/ml, 40 µl/h</th>
<th>1.5 mg/ml, 333 µl/h</th>
<th>12.5 mg/ml, 10 µl/h</th>
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</thead>
<tbody>
<tr>
<td>Drug delivery rate, µg/min</td>
<td>8.6</td>
<td>4.3</td>
<td>8.3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Total daily dose, mg</td>
<td>12</td>
<td>6</td>
<td>12</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Lumbar CSF concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of animals</td>
<td>10</td>
<td>5</td>
<td>3</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Lumbar [morphine], µg/ml</td>
<td>42 (20, 56)</td>
<td>19 (14, 30)</td>
<td>6 (5, 6)</td>
<td>42 (23, 56)</td>
<td>13 (10, 20)</td>
</tr>
<tr>
<td>Lumbar [Mor 3 Glu], ng/ml</td>
<td>128 (55, 380)</td>
<td>82 (82, 82)</td>
<td>1 (1, 2)</td>
<td>128 (26, 179)</td>
<td>0 (0, 77)</td>
</tr>
<tr>
<td>Lumbar [Mor 6 Glu], ng/ml</td>
<td>0 (0, 141)</td>
<td>23 (23, 23)</td>
<td>8 (4, 12)</td>
<td>145 (0, 184)</td>
<td>0 (0, 29)</td>
</tr>
</tbody>
</table>

Lumbar cerebrospinal fluid (CSF) sampled after 24–48 h of continuous infusion into the lumbar intrathecal space. Data are presented as median concentrations with quartiles.

Mor 3 Glu = morphine-3-glucoronide; Mor 6 Glu = morphine-6-glucoronide.

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infusion catheter. Several points were derived from these distribution studies: (1) At a fixed infusion rate over the range of 1.5–12.5 mg/ml at 40 μl/h (60–500 μg/h), there was a close correlation between infusion concentration (dose) and CSF concentrations at the sampling site 2–3 cm distant from the infusion site. Calculating the ratio of infusion concentration (in μg/ml) to that measured at the sampling site (table 4) for 12.5, 6, and 1.5 mg/ml, we found a similar ratio across the range of infusion dose/concentrations, e.g., 298, 316, and 250, respectively. This emphasizes that the rate of clearance over this distance is independent of dose with a fixed infusion rate. (2) The steepness of the concentration gradient from the tip is likely dependent on (a) the total dose delivered per unit time and (b) rate of infusion. Therefore, when 1.5 mg/ml was infused at 330 μl/h (e.g., 500 μg/h), the gradient was 36. Conversely, when 12.5 mg/ml was infused at 10 μl/h (125 μg), the gradient increased to 1,785. Therefore, rostrocaudal distribution over a distance as short as 3 cm varies directly with total dose and indirectly with rate.

As noted, granulomas of approximately 5 cm in length arose reliably with the 12.5 mg/ml at 40 μl/h infusion. When sampled at what would be the rostral edge of the granuloma, the 12.5 mg/ml at 40 μl/h (e.g., 500 μg/h) dose gave concentrations of approximately 50 μg/ml. Accordingly, we would indicate that in this model, local concentrations less than 50 μg/ml do not reliably lead to granulomas. This assertion is consistent with the observation that with the 1.5 mg/ml at 330 μl/h (e.g., 500 μg/h) dose, granulomas were also not observed, and the local concentration at 2–3 cm was 42 μg/ml. When 12.5 mg/ml at 10 μl/h (e.g., 125 μg/h) was examined, we noted that three of five dogs displayed masses of limited extent. We thus conclude that at the tip of the catheter, the dose of 3 mg/h in a small volume led to a local concentration that was indeed sufficient to initiate a spatially limited granuloma. This steep drug gradient likely accounts for the typically limited distribution of the granuloma even over extended periods of infusion in animals and humans. Therefore, in the current study, the longitudinal extent of the granuloma after 31 days did not greatly differ from that observed at 10 days, with a maximum length of approximately 5 cm. The mechanisms establishing this steep gradient in the face of continuous infusion must reflect dilution by the local CSF, but more importantly a local clearance. Previous work has shown that significant loss of opiates after intrathecal delivery occurs by movement into tissue, but perhaps more importantly into the large surface presented by the adjacent meninges.18

An important issue relates to the local morphine concentration that leads to granuloma formation. Although highly speculative, if we were to accept that the rostrocaudal distribution gradient is linear across doses, the actual concentrations near the infusion site might be estimated by multiplying the distant sampling concentration by the ratio of the calculated gradients measured at the low and high infusion rates (e.g., 298/36 = 8). Accordingly, for the 12.5- and 1.5-mg/ml infusates, the corrected local concentrations near the catheter tip with a 40-μl/h infusion would be 8 × 42 = 336 μg/ml and 8 × 6 = 48 μg/ml, respectively. As a caveat to the above comments, these data model a continuum of dilution and clearance between the two points (the tip and the sampling site). It is likely, given the complexity of spinal CSF kinetics, that there is considerable heterogeneity. Further, development of a small granuloma may itself result in altered flow patterns, which then impede redistribution.

The steepness of the observed gradient and the role of low infusion rates, even with continuous infusion, is consistent with the concepts previously expressed in human intrathecal studies considering the apparent decline in concentrations when observations (sampling) are made at a distance from the delivery site.19,20 Therefore, sampling at T-10 after a bolus delivery at L5–S1 of 50 μg morphine in 2 ml revealed maximum concentrations (275 ng/ml) at approximately 40 min after injection; this suggests a maximum gradient of 90-fold.21 In clinical cases, the concentration of morphine at the tip of a chronic intrathecal catheter has not been well documented, but when concentrations at the tip are assessed, they are typically increased. Bigler et al.22 reported concentrations of 240 μg/ml after bolus deliveries of 14 mg/ml when sampled from the injection catheter.

**Time Course of Granuloma**

**Onset.** The current studies emphasized that with serial MRI monitoring, all dogs displayed a detectable granuloma signal by 10 days after the initiation of infusion. Although these observations seem to suggest a more rapid time course than those observed in humans, the actual time frame of human granuloma formation must be considered uncertain for two reasons. First, it is clear that in dogs, the formulation of a granuloma over a 28-day interval is dose dependent. In humans, intrathecal therapy typically involves progressive increasing of doses over an extended period, and the addition of other agents may variably occur over the interval of therapy. Therefore, it is difficult to know when a triggering drug exposure is actually reached in the infused patient. This consideration raises an interesting question: In dogs, if a minimally effective dosing regimen (e.g., 1.5 mg/ml at 40 μl/h) were used for an extended time period (e.g., greater than 28 days), would the incidence of granuloma formation increase with time? Currently, we do not know the answer to this question. Further, it is unknown if there is a window of vulnerability, that once passed, later mass formation is unlikely. Second, with regard to the onset of the granuloma in the patient, diagnosis is
typically dependent on the appearance of some neurologic sign, which leads to imaging. As noted in these and previous preclinical studies,12,13 granuloma-associated neurologic signs may not be evident until well after the development of the mass. Therefore, in one case series of seven patients, one patient displayed neurologic symptoms, but computed tomography myelography of all patients revealed that the symptomatic patient and two additional asymptomatic patients displayed a granuloma (e.g., three of seven). Accordingly, the onset of neurologic signs may not be a true indication of the time of mass onset. Even with these caveats, four patients have been reported who had received infusion for less than 6 months before developing a granuloma of sufficient mass to produce neurologic signs.14

Regression. With regard to regression, there have been anecdotal comments that termination of infusion would lead to reversal of the granuloma in clinical cases. The current work clearly indicates that such a reversal does reliably occur over an interval of 7–14 days. Importantly, the reversal of the granuloma in the face of continued infusion of vehicle emphasizes that the effects are not the result of a reaction to the implant or the infusion. The degree of regression was greatest in a granuloma that was newly formed over 10 days. At longer intervals, the granuloma regression continued to occur but was less complete. Although not well characterized, consensus statements and reviews typically reflect a belief that the granuloma is reversible,23 but there are few systematic reports. In human patients displaying a morphine-related intrathecal granuloma, serial MRIs after removal of the morphine from the infusion indeed revealed regression of the mass and associated neurologic signs by 4 weeks.24 Whether long-term establishment of the granuloma reduces the reversibility as shown in the dogs has not been reported.

Conclusions

Using serial MRI analysis, the current studies emphasize the rapid formation and, importantly, the regression of the aseptic masses initiated by continuous intrathecal infusion of morphine. The current data strongly suggest an important role of local concentration. It is evident by direct lumbar CSF sampling that in dogs, there is an extraordinarily high concentration of drug near the catheter tip. These studies confirm the presence of a steep, longitudinal gradient from the catheter tip, even in the face of continuous infusion.

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