Pharmacokinetics and Efficacy of Epidurally Delivered Sustained-release Encapsulated Morphine in Dogs

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Background: We evaluated the epidural effects of a multivesicular liposome-based sustained-release preparation of morphine (C0401) on behavior and lumbar cerebrospinal fluid and serum kinetics of morphine.

Methods: Beagle dogs were prepared with lumbar epidural catheters with subcutaneous injection ports and lumbar intrathecal catheters. Each dog (n = 6) received the following by the epidural route: 5 mg/3 ml morphine sulfate in saline (MS-5), 10 mg/5 ml C0401 (C0401-10), and 30 mg/3 ml C0401 (C0401-30). Behavioral and physiologic parameters and nociceptive responses (skin twitch latency) were evaluated, and morphine concentrations were determined in lumbar cerebrospinal fluid and serum.

Results: All morphine treatments blocked the skin twitch response. Time to onset was 1.3 ± 0.3 h for C0401-30; 2.6 ± 0.6 h for C0401-10; and 4.4 ± 0.2 h for MS-5. Duration of action was 62 ± 0.3 h for C0401-30; 27 ± 2 h for MS-5. All treatments produced a modest reduction in arousal, muscle tone, and coordination, with the duration of the C0401-30 preparation being longer lasting. Respiratory rate was mildly depressed by all treatments, and moderate hypotension was noted. Time to peak cerebrospinal fluid morphine concentration was 11 h for C0401-30; 3 h for C0401-10; and 5 min for MS-5. Peak lumbar cerebrospinal fluid level was 34,992 ± 5,578 ng/ml for MS-5; 14,483 ± 5,389 ng/ml for C0401-30; and 10,730 ± 2,888 ng/ml for C0401-10. Morphine mean residence time in lumbar cerebrospinal fluid was 0.8 ± 0.1 h for MS-5; 8.9 ± 1.0 h for C0401-30.

Discussion: Kinetics studies showed that multivesicular liposome sequestration results in a restrained and persistent release of morphine from the epidural space. This extended release corresponded with an extended duration of analgesia without an attendant increase in the incidence of side effects. (Key words: Analgesia; cerebrospinal fluid; liposomes)

Epidural delivery of morphine is used to produce a potent analgesia in animals and humans that may persist for 6–12 h.¹² To achieve longer durations of action, a continuous infusion by catheter or use of a higher bolus dose can be used.³−⁵ Long-term infusion necessitates an available epidural catheter, and higher bolus dosing increases the amount of drug that may undergo redistribution, yielding undesirable side effects. Preparation of drugs in liposome vesicles permits the spinal delivery of larger absolute quantities and the retention of lower free concentrations of the agent.⁶−⁸ In the rat, epidural delivery of morphine encapsulated in multivesicular liposomes permitted delivery of large doses of morphine, which produced significant antinociception of an extended duration without an increase in the magnitude of side effects. In comparison, injection of high doses of morphine in saline extended the duration of action, but at the cost of a significant increase in the incidence and intensity of side effects.⁸

Previous work has been carried out largely in small-animal models and has not been accompanied by a systematic pharmacokinetic analysis. We sought in the current investigations to determine the behavioral (pharmacodynamic) and pharmacokinetic properties of morphine encapsulated in a proprietary, multivesicular liposomal preparation (DepoFoam™ drug-delivery system, Depotech Corp., San Diego, CA) of morphine after epidural delivery in dogs prepared with long-term lumbar intrathecal and epidural catheters.

Methods

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

Six male dogs were prepared with long-term lumbar intrathecal and epidural catheters. After a 3-day recovery period, dogs received an epidural injection of morphine sulfate in saline (5 mg) (MS-5), followed 2 days later by an epidural injection of 10 or 30 mg C0401 (C0401-10, C0401-30; Depotech Corp.). The dogs subsequently received a second epidural injection of C0401 (10 or 30 mg) in crossover fashion 10 days later. Venous blood and lumbar cerebrospinal fluid (CSF) were sampled at intervals after each drug injection. An additional group of five dogs was prepared to receive the DepoFoam vehicle alone. In each case, blood pressure, heart rate, respiratory rate, and skin-twitch response latencies were assessed periodically before and after the EPI injection.

Animals

Male beagle dogs were obtained from Harlan Sprague Dawley (Ridgian Farms, Mount Hope, WI). The animals were approximately 8–12 months old (sexually mature) at initiation of treatment and weighed 8.4–12.8 kg. Dogs were housed in individual runs in an American Association for the Accreditation of Laboratory Animal Care accredited vivarium. Water and food (Certified Teklad 25% Lab Dog Diet, Harlan Teklad, Madison, WI) were provided, except during food fasting periods before surgery and necropsy. Dogs were accommodated to the laboratory environment for a minimum of 5 days before the start of the study. During the acclimation period, animals underwent physical examination and were defined as being in good health on the basis of physical examination and determination of hematology and clinical values (performed by Biomedical Testing Services, San Diego, CA). These values were all within normal range for beagles (data not shown).

Epidural/Intrathecal Catheter Placement

To permit sampling of lumbar CSF and epidural drug delivery, lumbar intrathecal and epidural catheters were placed in a single surgery. After adaptation and health certification, dogs underwent implantation. Order of treatment was assigned on the basis of order of arrival at the vivarium. A prophylactic treatment of sulfamethoxazole–trimethoprim tablets (15–23 mg/kg oral twice daily, 480 mg/tablet) was started on day-5 and continued through day-1. On day-3, after an overnight fast, dogs received atropine (0.4 mg/kg, intramuscular) followed by ketamine hydrochloric acid (1 mg/kg) and diazepam (0.5 mg/kg) delivered intravenously. The trachea was intubated and anesthesia was maintained with spontaneous ventilation of 1 to 2% halothane and 60% nitrous oxide–40% oxygen. Animals were continuously monitored for oxygen saturation; inspired and end-tidal values of halothane, carbon dioxide, nitrous oxide, and oxygen; and heart and respiratory rates. Surgical areas were shaved and surgically prepared, and the animals were draped.

To place the intrathecal catheter, the cisternal membrane was exposed by a cutaneous incision on the back of the head and by blunt dissection.10 A polyethylene PE50 catheter then was passed 40 cm caudally in the intrathecal space to lie at the L1–2 level. The catheter was externalized on the upper back. The animal was redraped and the epidural catheter was placed.

To catheterize the lumbar epidural space, a skin incision (2 cm) was made over the L7–S1 interspace, and an 18-gauge Tuohy needle was passed through the incision into the epidural space. A PE50 catheter then was passed to lie approximately 10 cm within the epidural space at approximately the L1–2 level. Appropriate placement of the catheter tip was determined by observation of negative pressure in the needle during inspiration by the dog and by absence of CSF outflow from the catheter. The portion of the catheter external to the epidural space was connected to a subcutaneous injection port secured at the lower back. At closure of the incisions, the anesthesia gases were turned off and the animal was observed during recovery. Butorphanol tartrate (Torbugesic®; Fort Dodge Animal Health, Fort Dodge, IA; 0.04–0.1 mg/kg, intramuscular) was administered to all animals to relieve postoperative pain. No animals needed more than a single injection.

Test Articles

Sustained-release encapsulated morphine (C0401) was prepared using a double-emulsification procedure,11 modified for aseptic and scaled-up manufacture. Briefly, aqueous morphine sulfate solution was emulsified with a solution of lipids in chloroform. The first emulsion (“water in oil”) was transferred to a second emulsification vessel, along with a solution of dextrose and lysine, and subjected to high-speed shear to create a “water in oil in water”–type double emulsion. The second emulsion then was sparged with nitrogen gas to remove chloroform, resulting in the formation of lipid-based (DepoFoam) particles containing entrapped morphine solution. The particles were washed free of nontencapsulated drug and lipid and the dextrose-lysine solution was exchanged for saline by cross-flow filtration. The drug concentration of the suspension was adjusted to the
desired concentration of active ingredient by either addition of saline or decanting of settled particle-free supernatant solution. Vials (2 ml) were filled aseptically with a homogenous suspension, stopper-sealed, and stored under refrigeration.

The C0401 test article used in the current investigation contained morphine at a nominal concentration of 10 mg/ml, with less than 5% of the total morphine in the formulation present as unencapsulated drug. Lipids comprising the DepoFoam matrix included 1,2-diocetyl-sn-glycero-3-phosphocholine (4.3 mg/ml), 1,2-dipalmityl-sn-glycero-3-phospho-rac-(1-glycerol), sodium salt (0.8 mg/ml), cholesterol (3.2 mg/ml), triolein (0.1 mg/ml), and tricaprylin (0.3 mg/ml). The mean particle diameter was 14.7 μm, and the pH of the suspension was 7.5. The DepoFoam vehicle control article was prepared similarly, with the exception that sucrose was used in the first aqueous solution instead of morphine sulfate. Morphine sulfate was dissolved in 0.9% (w/v) sodium chloride for injection USP.

Drug Preparation and Delivery
C0401 test article and DepoFoam vehicle control article were resuspended by gentle mixing. These agents and morphine sulfate in saline were delivered epidurally in a volume of 3 ml over an interval of 60-120 s. Each injection was followed by 0.8 ml saline control vehicle to clear the injection port and catheter of the test or vehicle control article.

Study Measures
Behavioral Observations. Dogs were observed by the investigators at the intervals described herein by the investigators. A description of the scoring procedures for behavioral function (arousal, muscle tone, and coordination) is given elsewhere[11] but involves assigning a rating of 0, 1, 2, or 3 to reflect increasing (+) or decreasing (−) characteristics, with 0 being normal.

Skin Twitch. The thermally evoked skin-twitch response was measured using a probe with approximately 1 cm of surface area maintained at approximately 62.5°C with a feedback controller. The probe was applied to shaven thoracic and lumbar areas of the back. Depression of the probe on the skin activates a timing circuit that is stopped when the probe is removed. Typically, placement of the probe results in a clear, brisk contraction of the local paraspinal cutaneous musculature within 1-3 s of the probe placement. At appearance of this response, the probe was removed and the latency was recorded. Failure to respond within 6 s was cause to remove the probe and assign that value (6 s) as the latency. Significant antinociception was defined as an increase of two standard deviations above preinjection baseline, with the standard deviation based on the overall group preinjection data.

Physiologic Parameters. Heart rate (beats/min) and blood pressures (mmHg) for each animal were measured using a tail-cuff manometer (Dinamap® 8100; Critikon, Inc., Tampa, FL). Respiration rate (breaths/min) was measured by observation of chest expansion and contraction. Rectal temperature (°C) was assessed using a digital rectal thermometer placed 5 cm rectally.

Timing of Observations and Sampling
Based on preliminary experiments defining duration and drug kinetics, the animals were observed for 2 days after M5-5, 5 days after C0401-10, and 7 days after C0401-30.

With each injection, skin twitch and behavioral and physiologic functions were assessed predose (15 min before injection) and postdose (15, 30, 60, 120, 180, 240, 300, and 600 min, 24 h, and daily thereafter for the remainder of the dosing interval). To define the kinetics of morphine, lumbar CSF and serum samples were taken predose (20 min before injection) and postdose (2, 5, 20, 40, 80, 160, 320, and 640 min, 24 h, and daily thereafter for the remainder of the dosing interval).

Sample Handling
Blood (approximately 2 ml) and lumbar CSF (approximately 0.3 ml) samples were taken immediately before and at intervals after the epidural injection. Blood samples were collected on wet ice and prepared as serum in red-top vials. CSF samples were collected on wet ice and split into duplicate samples, and both serum and CSF were stored frozen at −20 ± 10°C.

Morphine Assay
Morphine concentrations in serum and CSF were determined by radioimmunoassay using a commercially available kit (Coat-A-Count™ Serum Morphine, Diagnostic Products Corporation, Los Angeles, CA). This kit is specific to morphine and does not cross-react with glucuronide conjugates. Kits were validated using dog serum and CSF. The limits of quantitation and detection of the assay were 5.0 and 2.5 ng/ml, respectively. The assay displayed linearity over the range of 5–250 ng/ml. Samples were diluted with saline as necessary to use the linear portion of the assay. A calibration curve using

Anesthesiology. V 90, No 5, May 1999
morphine standards spiked into the appropriate matrix was included in each assay.

Necropsy
At completion of the study, each animal was anesthetized with sodium pentobarbital (Nembutal® Pentobarbital Sodium for injection; Abbott Laboratories, North Chicago, IL; 35 mg/kg, intravenously). The spinal cord dura was exposed by laminectomy of the spinal canal and the lower brain stem was exposed. Evan’s blue dye was injected through each catheter to confirm catheter integrity, position, and dye spread.

Statistical Analyses
Continuous normal data were compared using repeated measures and one-way analysis of variance with post hoc Bonferroni tests for multigroup comparisons. Distribution-free testing of data, such as ranked observations, was performed using the Kruskal-Wallis test for comparison of three or more groups with post hoc Mann-Whitney U tests for multigroup comparisons. All statistical comparisons were made at the \( P < 0.05 \) level of significance. For cross-treatment comparisons of behavioral data, the area under the curve (AUC) was calculated from time of injection to 48 h.

Morphine levels in serum and lumbar CSF were plotted as the mean and SEM as a function of time after each test article dose. Assay values below the defined detection limit of the assay (2.5 ng/ml) were not included in the kinetic analysis, but are used in the graphic presentation. Noncompartamental pharmacokinetic analyses (WinNonLin; Scientific Consulting, Inc., Apex, NC) were performed to determine time to maximum concentration \( (T_{\text{max}}) \), maximum concentration \( (C_{\text{max}}) \), elimination half-life \( (t_{1/2}) \), AUC, area under the first moment curve (AUMC), and mean residence time (MRT). Analyses were performed in parallel on the curves from each animal to determine the parameters. The results from each animal were used to define a mean and SEM for each parameter. In addition, parallel analysis was performed on mean morphine serum and CSF concentration–time curves.

Results
All animals survived until the scheduled date of necropsy and received all scheduled treatments. At the time of killing, inspection of the epidural and intrathecal catheters revealed that all were intact and patent. Identification of the location of the epidural catheters revealed that all were located in the epidural space, with the tip between L2 and T13. The intrathecal catheter tips were identified as being located between 1 and 2 cm from the epidural catheter tip.

General Behavior
Epidural injection of MS-5, C0401-10, or C0401-30 did not evoke any signs of agitation or discomfort. In a separate group of five dogs receiving DepoFoam vehicle alone, there was no evidence of agitation or discomfort.

Epidural MS-5 produced rapid (peak at \(< 60 \) min) but moderate reductions in arousal, muscle tone, and coordination (fig. 1). These changes lasted for a period of 3–6 h after injection. C0401-10 and C0401-30 produced similar modest changes in arousal and muscle tone, but these changes occurred with a somewhat greater delay (3–6 h) and persisted 10–24 h after injection. Although the absolute peak effects were not different, the duration of the effects, as defined by the AUC, was dose-dependent (fig. 1).

Antinociception
All dose treatments produced a complete block of the skin-twitch response, i.e., response latency of 6 s or more (fig. 2). As indicated in figure 2, the ordering of time to onset of significant antinociception (i.e., skin twitch ≥ baseline ± 2 SD), from longest to shortest was blank DepoFoam (no effect up to 48 h) > C0401-30 > C0401-10 > MS-5 (\( P < 0.05 \); fig. 2, bottom left). With regard to duration of antinociception, the rank order of time to offset from longest to shortest was: C0401-30 > C0401-10 = MS-5 > blank DepoFoam (\( P < 0.05 \); fig. 2, bottom right).

Physiologic Function
Heart rate monitoring displayed a moderate bradycardia, which was comparable for all drugs, although onset of depression occurred immediately after injection of MS-5 compared with 2 h after injection of C0401-10 and C0401-30 (fig. 3). Moderate decreases in mean systolic and diastolic blood pressures also were noted, with similar time courses. There was a moderate decrease in respiratory rate after all treatments (data not shown). Low body temperatures were noted at 24 h after C0401-10 and C0401-30 injection but recovered to baseline within 48 h after test article delivery (data not shown). Body temperatures were normal in dogs at 24 h after MS-5 injection; however, a previous decrease may have been missed because 24 h was the earliest measurement.
Pharmacokinetics

Morphine concentrations in lumbar CSF are shown in figure 4. Morphine levels peaked rapidly (within 5–10 min) in lumbar CSF after epidural administration of MS-5. Concentrations decreased quickly thereafter to below the detection limit (2.5 ng/ml) within 24–48 h. In contrast, morphine concentrations did not reach a peak in lumbar CSF until 3–11 h after administration of C0401-10 or C0401-30. Peak morphine concentrations were approximately threefold lower after C0401-30 administration, compared with MS administration at one sixth the dose. After reaching peak levels after C0401 dosing, the morphine concentrations subsequently decreased essentially in first order in the CSF. This “elimination phase” was roughly parallel for C0401-10 and C0401-30 administration. The morphine concentrations in lumbar CSF did not return to baseline until 120 and 144 h for C0401-10 and C0401-30 administration, respectively.

Morphine concentrations in serum are shown in figure 4. Morphine pharmacokinetic profiles in serum were similar to those observed in lumbar CSF, with the exception that serum levels were approximately 150- to 300-fold lower at the peak. After administration of C0401-10 and C0401-30, morphine concentrations returned to the lower detection limits of the assay (baseline) by 24 and 72 h, respectively, in the majority of animals (four of six), in contrast to a 5-h return to the lower detection limits of the assay in four of six animals after MS-5 administration.

A summary of the noncompartmental pharmacokinetic analysis is reported in table 1. As indicated, there were no evident differences in the results whether calculated for each individual animal or for the group mean concentrations. The mean residence times of morphine in both the lumbar CSF and the serum were increased approximately 17-fold after C0401-30 administration, compared with administration of MS-5 (13–14 h vs. 45–60 min). Morphine kinetics were
Fig. 2. (Top) Time-dependent changes in skin twitch after epidural injection of 5 mg/3 ml morphine in saline, 10 mg/3 ml C0401, 30 mg/3 ml C0401, or blank DepoFoam (3 ml). Values are presented as the mean ± SD of the respective response latencies. N = five animals at each treatment. The rank ordering of the absolute peak increase in skin-twitch response latency was 30 mg/3 ml C0401 > 10 mg/3 ml C0401 = 5 mg/3 ml morphine in saline > blank = 0 (one-way repeated measures analysis of variance, P < 0.05 and post hoc Bonferroni tests). (Bottom) Histograms present the time of onset (left) and offset (right), respectively, of antinociceptive effects of epidurally administered blank DepoFoam (B); epidural morphine (5 mg in saline; MS-5), 10 mg/3 ml C0401 (C-10) or 30 mg/3 ml C0401 (C-30). The ordering of the time to onset was 10 mg/3 ml C0401 = 30 mg/3 ml C0401 > 5 mg/3 ml morphine in saline (one-way repeated measures analysis of variance, P < 0.05 and post hoc Bonferroni tests). The ordering of the time to offset was 30 mg/3 ml C0401 = 10 mg/3 ml C0401 = 5 mg/3 ml morphine in saline (one-way repeated measures analysis of variance, P < 0.05 and post hoc Bonferroni tests). Blank DepoFoam injections had no significant effect on nociceptive thresholds.

essentially linear in the serum, because the AUC ratios were roughly equivalent to the dose ratios. However, the data indicate that the bioavailability of morphine in the lumbar CSF, and presumably at the spinal dorsal horn sites, may have been enhanced by delivery in the DepoFoam formulation because the AUC ratios were two- to threefold greater than the dose ratios when comparing C0401 with MS-5 administration (dose ratio = 1.2:6; serum AUC ratio = 1:2.5:8.2; lumbar AUC ratio = 1:6.4:13.4; data based on group AUC values in table 1).

Discussion

The current studies show that the encapsulation of morphine in a multivesicular liposome preparation (C0401) results in a controlled release of the encapsulated material. Thus, epidural delivery of C0401-30 resulted in peak lumbar CSF levels that were threefold less than those produced by MS-5 and an extended CSF mean residence time (17-fold increase). Consis-

tent with these pharmacokinetics, C0401-30 displayed an antinociception that was no less intense than MS-5 but, in contrast, showed an extended duration. Importantly, despite the persistent antinociception and consistent with the controlled release, side effects after epidural C0401 were no more intense than those produced by MS-5. Several issues may be considered pertinent.

Model

The long-term epidural catheter model for the evaluation of test article pharmacology and safety has been used for bolus epidural drug delivery.12 The placement of the catheter in the epidural space was confirmed at placement (negative pressure at insertion and absence of blood or CSF) and at necropsy by the physical presence of the catheter and distribution of dye in the epidural space. By these criteria, all catheters were determined to be freely patent and in the epidural space for the duration of the study.

Anesthesiology, V 90, No 5, May 1999
HEART RATE

MEAN ART PRESSURE

SYSTOLIC PRESSURE

DIASTOLIC PRESSURE

TIME (Hours)

Fig. 3. Time-dependent changes in heart rate and mean systolic and diastolic blood pressure after epidural injection of blank DepoFoam, 5 mg/3 ml morphine in saline, 10 mg/3 ml C0401, or 30 mg/3 ml C0401. Measures are presented as the mean ± SD of the respective physiologic measurements (N = six animals at each treatment). The ordering of the magnitude of the bradycardia and depression of mean arterial blood pressure and systolic and diastolic blood pressures was 30 mg/3 ml C0401 = 10 mg/3 ml C0401 = 5 mg/3 ml morphine in saline > blank = 0 (one-way analysis of variance, \( P < 0.05 \) and post hoc Bonferroni tests).

Antinoceptive

The measure of noception used in the current studies, the skin-twitch response, is a thermally evoked, C-fiber-mediated polysynaptic nociceptive reflex.\textsuperscript{13} Considerable evidence has shown that this response is modulated by a direct spinal action of \( \mu \)-opioid agonists.\textsuperscript{14,15}

In the superficial dorsal horn, opiate agonists can interact with \( \mu \)-opioid receptors located preterminally on C-fibers and postsynaptically on dorsal horn projection neurons. This local action serves to reduce the release of small afferent transmitters and to hyperpolarize dorsal horn neurons. These joint effects are believed to account for the selective block of spinal nociceptive transmission.\textsuperscript{16} The time to onset was fastest after MS-5 and delayed by approximately 2 h after delivery of the encapsulated material, an observation consistent with a delayed movement of morphine from the epidurally delivered liposomes.

Behavioral Effects

A modest reduction in arousal was observed after injection of MS-5 and C0401 test articles. The effects on arousal are mediated by a supraspinal redistribution of the spinally delivered morphine in dogs.\textsuperscript{12,17} The reductions in hind limb muscle tone and motor coordination

Fig. 4. Concentrations of morphine in lumbar CSF (top) and serum (bottom) as a function of time after the epidural delivery of 5 mg/3 ml morphine in saline (MS-5); 10 mg/3 ml C0401 (C0401-10), or 30 mg/3 ml C0401 (C0401-30). Data are presented as the mean ± SEM (N = five animals at each treatment). See table 1 for kinetic analysis.
Table 1. Pharmacokinetic Analysis of Morphine in Lumbar CSF and Serum after Epidural Delivery of Morphine (5 mg) in Saline (MS-5) or C0401 (10 mg) or C0401 (30 mg)

<table>
<thead>
<tr>
<th></th>
<th>Lumbar CSF</th>
<th>Serum</th>
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</thead>
<tbody>
<tr>
<td>MS-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(T_{\text{MAX}}) (h)</td>
<td>N/A (0.08)†</td>
<td>N/A (0.03)</td>
</tr>
<tr>
<td>(C_{\text{MAX}}) (ng/ml)</td>
<td>34,942 ± 5578 (34053)</td>
<td>230 ± 30 (230)</td>
</tr>
<tr>
<td>(T_{1/2}) (h)</td>
<td>3.5 ± 0.2 (3.5)</td>
<td>0.8 ± 0.2 (0.9)</td>
</tr>
<tr>
<td>(AUC_{0-\infty}) (ng · h/ml)</td>
<td>17,682 ± 1,440 (17681)</td>
<td>129 ± 16 (130)</td>
</tr>
<tr>
<td>(AUMC_{0-\infty}) (ng · h²/ml)</td>
<td>13,307 ± 1,547 (13,303)</td>
<td>134 ± 38 (129)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>0.8 ± 0.1 (0.8)</td>
<td>0.9 ± 0.2 (1.0)</td>
</tr>
<tr>
<td>C0401-10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(T_{\text{MAX}}) (h)</td>
<td>N/A (2.7)</td>
<td>N/A (2.7)</td>
</tr>
<tr>
<td>(C_{\text{MAX}}) (ng/ml)</td>
<td>10,730 ± 2,886 (8,901)</td>
<td>35 ± 7 (31)</td>
</tr>
<tr>
<td>(T_{1/2}) (h)</td>
<td>7.5 ± 0.4 (8.0)</td>
<td>4.1 ± 0.6 (4.3)</td>
</tr>
<tr>
<td>(AUC_{0-\infty}) (ng · h/ml)</td>
<td>163,181 ± 65,336 (113,579)</td>
<td>312 ± 31 (319)</td>
</tr>
<tr>
<td>(AUMC_{0-\infty}) (ng · h²/ml)</td>
<td>1,552,695 ± 696,600 (1,030,580)</td>
<td>2,416 ± 426 (2311)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>8.9 ± 0.8 (9.1)</td>
<td>7.5 ± 0.7 (7.3)</td>
</tr>
<tr>
<td>C0401-30</td>
<td></td>
<td></td>
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<tr>
<td>(T_{\text{MAX}}) (h)</td>
<td>N/A (10.67)</td>
<td>N/A (10.7)</td>
</tr>
<tr>
<td>(C_{\text{MAX}}) (ng/ml)</td>
<td>14,483 ± 3,438 (12,821)</td>
<td>59.1 ± 4.8 (52.5)</td>
</tr>
<tr>
<td>(T_{1/2}) (h)</td>
<td>10.0 ± 0.6 (10.0)</td>
<td>10.7 ± 2.2 (9.4)</td>
</tr>
<tr>
<td>(AUC_{0-\infty}) (ng · h/ml)</td>
<td>236,998 ± 39,750 (237,148)</td>
<td>1,100 ± 90 (1069)</td>
</tr>
<tr>
<td>(AUMC_{0-\infty}) (ng · h²/ml)</td>
<td>3,074,651 ± 609,508 (3,099,920)</td>
<td>17,779 ± 3,473 (15,275)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>12.8 ± 1.0 (13.1)</td>
<td>16.2 ± 3.0 (14.3)</td>
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\(T_{\text{MAX}}\) = time to peak concentration; \(C_{\text{MAX}}\) = peak concentration; \(T_{1/2}\) = elimination half-life; \(AUC_{0-\infty}\) = area under concentration versus time curve from time 0 to infinity; \(AUMC_{0-\infty}\) = area under first moment curve from time 0 to infinity; MRT = mean residence time (AUMC_{0-\infty} / \(AUC_{0-\infty}\)); N/A = not applicable.

* Mean ± SEM of parameter calculated for each dog (N = 3–6).
† PK calculated using group mean curve.

were transient and probably reflect in part the sedative effect of the high delivered dose and a direct effect on motor horn cells. Although it is not commonly appreciated, \(\mu\) opiates hyperpolarize motor neurons and can produce a moderate reduction in motor outflow.16

**Physiologic Effects**

Epidural morphine given in saline and in the encapsulated form resulted in apparent but not physiologically compromising or life-threatening effects.

**Blood Pressure.** Epidural injection of MS-5, C0401-10, or C0401-30 resulted in a modest reduction in heart rate and blood pressures. The reduced blood pressure may reflect reduced peripheral resistance caused by reduced sympathetic outflow or vasodilatation secondary to systemic histamine release caused by circulating morphine.17 Spinal \(\mu\) opiates are not considered to have significantly effects on preganglionic sympathetic outflow.15,18,19

**Rectal Temperature.** Temperature showed a prominent decrease at 24 h after epidural C0401, but not after MS. The absence of effect of MS-5 is likely due to the fact that temperature was measured only at 24 h, when free morphine levels had significantly decreased. Low doses of morphine also can induce transient bouts of panting,20 which can depress core temperature.21,22

**Respiration.** Opiates, by an action on brain stem neurons, depress the carbon dioxide drive and, accordingly, tidal volume.23 Respiratory rate was mildly depressed by the epidural agents at the doses used. This suggests there was relatively minimal redistribution of morphine when given at the dose of 5 mg in saline or at doses as high as 30 mg in the C0401-30 formulation.

**Pharmacokinetics**

The spinal effects of epidurally delivered morphine result from the movement of the agent from the epidural space into the spinal parenchyma secondary to movement through the local meninges. Although it has been hypothesized that the movement also may occur secondary to absorption in local radicular arteries and veins, findings of studies of parenchymal redistribution have not supported that speculation.24 In addition to transmeningeal diffusion, epidural drugs are exposed to the thin-walled epidural venous plexus (Batson’s plexus), which then drains via the ayzygos vein into the vena cava and to the lymphatic drainage.25,26

In the current studies, after epidural injection of MS-5,
morphine displayed a rapid peak appearance (< 5 min) in lumbar CSF and an exponential decrease with a terminal half-life of 3.5 h. Evaluation of the serum levels revealed a similar time course with the peak slightly delayed, but with peak plasma concentrations being approximately one-fiftieth of those observed in the CSF. These properties are consistent with previous studies performed with epidural morphine in dogs and in humans after epidural injection. This early appearance of epidurally delivered drug in the systemic circulation may reflect the rapid local absorption in the epidural venous plexus. Thus, plasma concentration in the azygos vein of morphine after epidural delivery in dogs has been shown to be 5–10 times higher in azygos vein blood than in concurrently sampled arterial blood during the first hour after injection.

Evaluating the redistribution of CO401 morphine, it is clear that this distribution displayed a restrained distribution profile. First, in comparison with MS-5, for CO401-10 and CO401-30 peak CSF and serum concentrations were observed after longer intervals (3 and 11 h, respectively), and the lumbar CSF $T_{1/2}$ was increased (8 and 10 h, respectively). Second, evaluation of peak CSF concentrations revealed that despite delivering six times the total dose, the peak CSF concentrations observed after CO401-30 were approximately one third those of MS-5. In addition, the delay in onset of effects after administration of both doses of CO401 and the failure to see greater absolute effects on behavioral or cardiovascular indices compared with MS, in conjunction with pharmacokinetic observations, indicate that the CO401 formulation was associated with a titrated release of active drug.

Evaluation of the ratio of areas under the serum clearance curves for MS-5, CO401-10, and CO401-30 yielded values of 1.25:8.2, approximately corresponding to the ratio of total dose (e.g., 5, 10, and 30 mg morphine). These ratios would be expected because the blood represents the final common pathway for clearance of all epidurally delivered agents. In contrast, the same ratio calculated for the AUC of morphine in the lumbar CSF was 1.64:13.4. These data suggest that liposomal incorporation served to facilitate the movement of encapsulated morphine into the CSF. Morphine passes the meningeal barrier more slowly than agents such as alfentanil, in part because of its hydrophilicity and low lipid partition coefficient. We speculate that lipid encapsulation with the CO401 vehicle may facilitate passage of the hydrophilic morphine through the meningeal barrier and thus enhance its CSF bioavailability.

**Liposomes**

Several reports have used liposomes either intrathecally or epidurally to deliver opiates, oxycodone, local anesthetics, and chemotherapeutic agents. The premise is that this encapsulation creates a depot that provides a controlled release of agent into the biophase, which is available for redistribution. In the articles cited herein and in the current study, such "diffusion modifier" formulations allow single injections, with high doses being released slowly to provide an extended exposure. Because the sequestered material is not available for immediate redistribution, the peak concentrations of free drug are minimized, and side effects relative to the dose delivered are reduced. Such was the case in the current study, in which the prolonged duration of antinociceptive action was not accomplished by an increase in peak incidence or intensity of side effects.

The important variables defining liposome function are particle size, lipid constituents, particle structure (e.g., multilamellar vs. unilamellar), the drug-to-lipid load, and the potential toxicity of the liposome constituents. The DepoFoam drug-delivery system is a proprietary multivesicular liposomal preparation for providing controlled, sustained release of therapeutic agents. Multivesicular liposomes differ from more conventional liposomes in that the lipid particles are substantially larger (mean diameter of approximately 10–20 μm compared with <1 μm for unilamellar liposomes) and are composed of numerous nonconcentric internal aqueous chambers containing the encapsulated drug. The larger size of the DepoFoam particles and their multivesicular nature allow for high drug loading and use as a "drug depot" when injected into various body compartments, such as subcutaneous, intrathecal, or epidural spaces. DepoFoam particles apparently release drug over an extended period of time in vivo by gradual breakdown or reorganization of the lipid membranes. The lipid components of DepoFoam particles are synthetic duplicates of common, naturally occurring lipids and are expected to enter standard catabolic pathways after breakdown of the particles in vivo. Previous work has shown that encapsulation of morphine into a DepoFoam preparation produced an extended-release product after epidural delivery in a rodent model, with an extended duration of antinociceptive activity and a lower potential for extraspinal effects compared with equianalgesic doses of standard morphine. The sequestering efficiency is such that reliable high retention fractions can be achieved with a small total lipid load (e.g., <5% free agent with 10 mg morphine/11 mg lipid/ml). Such high loading has...
been an important characteristic of multivesicular preparations. It is difficult to compare these figures with other preparations because of insufficient information concerning methodology and because molecules differing in molecular weight or lipid solubility are differentially retained.

With regard to release rates, in unpublished studies (F. Kohn and T. L. Yaksh), we demonstrated the ability to systematically increase and retard release rates of morphine by modest alterations in membrane lipid compositions with this multivesicular preparation. Thus, manipulations that serve to "harden" the lipid bilayer decrease the fractional release rates in the dog model used in this study. The particle preparation used in this report was chosen on the basis of providing an effective CSF morphine concentration for an approximately 48-h interval of antinociception. As such, this preparation is targeted clinically as a single-dose postoperative pain medication.

An important issue relates to safety. In other studies, we showed that high concentrations of L-dipalmityloxyphosphatidylcholine but not D-dipalmityloxyphosphatidylcholine in the rat evoke agitation behavior. The relative load of lipids given in those experiments (1 mg) intrathecally was greater than the individual lipids used in the current study after epidural delivery in the dog. In the current study no evidence of motor dysfunction or agitation was noted in the blank DepoFoam formulation. In other work, four sequential injections of DepoFoam and DepoFoam morphine at 8-day intervals, with the animals being sacrificed for histopathology 24 h after the last injection, showed no effect of DepoFoam vehicle and no changes in CSF protein taken at that time.

With regard to clearance of the liposome itself, there are few systematic data. In recent studies, we showed that after intrathecal delivery of DepoFoam particles prepared with 14C-dioleoylphosphatidylcholine in rats, the majority of the radiolabel is cleared from the animal within an interval of 2 or 3 weeks. A small amount of the radioactivity was recovered from the central nervous system or peripheral tissues. It was argued that after breakdown of the lipid particles, liberated 14C-dioleoylphosphatidylcholine enter standard catabolic pathways, resulting in expiration of the label as 14CO2.

In conclusion, we believe that liposome formulations appropriately tested and defined represent an important method for systemically increasing the load of agent that can be delivered into the epidural space without increasing the amount of freely diffusible material. The current studies suggest that the present multivesicular liposomal preparation can reliably achieve such an aim with morphine (and other agents) given by an epidural route. Technically, the epidural site appears to be an ideal target for this sort of delivery system because it provides a physical sequestration of the injectate.

References


