Journal of the American Association for Laboratory Animal Science
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Vol 50, No 4
July 2011
Pages 507–512

Epidural Administration of Liposome-Encapsulated Hydromorphone Provides Extended Analgesia in a Rodent Model of Stifle Arthritis

Jennifer R Schmidt,[1] Lisa Krugner-Higby,[1] Timothy D Heath,[2] Ruth Sullivan,[3] and Lesley J Smith[1,*]

Liposome encapsulation of opioids by using an ammonium-sulfate–gradient loading technique significantly slows the release time of the drug. This study evaluated the duration of analgesia in a rodent model of monoarthritis after epidural administration of liposome-encapsulated hydromorphone (LE-hydromorphone; prepared by ammonium-sulfate–gradient loading) compared with standard hydromorphone and a negative control of blank liposomes. Analgesia was assessed by changes in thermal withdrawal latency, relative weight-bearing, and subjective behavioral scoring. Analgesia in arthritic rats was short-lived after epidural hydromorphone; increases in pain threshold were observed only at 2 h after administration. In contrast, thermal pain thresholds after epidural LE-hydromorphone were increased for as long as 72 h, and subjective lameness scores were lower for as long as 96 h after epidural administration. Injection of LE-hydromorphone epidurally was associated with various mild changes in CNS behavior, and 2 rats succumbed to respiratory depression and death. In conclusion, LE-hydromorphone prolonged the duration of epidural analgesia compared with the standard formulation of hydromorphone, but CNS side effects warrant careful administration of this LE-hydromorphone in future studies.

Abbreviations: LE-hydromorphone, ammonium-sulfate–gradient-loaded liposome-encapsulated hydromorphone; CFA, complete Freund adjuvant.

In laboratory animal medicine, frequent dosing of analgesics to large colonies of research animals—whether they are rodents, primates, dogs, or other species—imposes many challenges that may result in insufficiently frequent analgesic administration. Of these challenges, frequent animal handling increases animal stress and disease risk, as well as the risk of human exposure to zoonotic disease. In addition, in a funding environment with limited research dollars, the cost for additional personnel to administer multiple doses of analgesics may fall outside of a practical budget plan. These factors have spurred an ongoing search for longer acting and effective analgesics that can be prescribed at extended dosing intervals yet provide steady-state analgesia without untoward side effects.

Opioids are the most effective class of analgesic drugs available for the treatment of acute and some types of chronic pain,[9,20] although opioids are not considered 'first-line' drugs for the treatment of chronic neuropathic pain.[5] Compared with systemic dosing, epidural administration of opioids generally increases the duration of analgesia, although duration is still limited to hours. For example, in dogs, systemic morphine administered at 1.0 mg/kg IM provides analgesia for approximately 4 to 6 h,[9] whereas epidural morphine administration at 0.1 mg/kg provides analgesia for 12 to 24 h.[27] In people, a single low dose of epidural morphine provides analgesia for 6 to 12 h;[16] increasing the dose may afford a maximum of 12 additional hours of analgesia.[16,26] Lumbosacral epidural administration of certain opioids (for example, morphine) decreases systemic side effects, including sedation, pruritis, nausea, and vomiting, while providing adequate postoperative hindlimb analgesia.[30]

Liposome encapsulation of opioids may further increase the drug's duration of analgesia by providing a slowly released reservoir of drug within the liposomes. Depodur, a preparation of liposome-encapsulated morphine sulfate, has been shown to provide 48 h of analgesia when administered epidurally to people.[14,31,32,35,36] The Depodur product is approved by the Food and Drug Administration and is commercially available for people. A different method of loading liposomes with opioid drugs—using an ammonium sulfate gradient—results in a liposome-encapsulated opioid that, when administered subcutaneously, extends release for as long as 21 d in rhesus monkeys.[15]

The purpose of the current study was to assess the analgesic duration of action of ammonium-sulfate–gradient-loaded liposome-encapsulated hydromorphone (LE-hydromorphone) administered epidurally in a rat model of monoarthritis. Our hypothesis was that LE-hydromorphone would provide significantly longer analgesia in this model than would either a positive control using standard hydromorphone or a negative control using empty liposomes administered epidurally.

Materials and Methods

All experimental protocols were approved by the University of Wisconsin School of Veterinary Medicine Animal Care and Use Committee and adhered to the *Guide on the Use of Animals in Research*.[13] Any animal that exhibited signs of severe pain (autotomy, anorexia, lack of grooming, excessive porphyrin staining, lack of food or water intake) or polyarthritis would have been removed from the study and euthanized; in the current study, none of the rats required euthanasia.

Received: 10 Sep 2010. Revision requested: 13 Oct 2010. Accepted: 24 Nov 2010.
[1]Department of Surgical Sciences, School of Veterinary Medicine, [2]School of Pharmacy, and [3]Research Animal Resources Center, University of Wisconsin, Madison, Wisconsin.
[*]Corresponding author. Email: smithl@svm.vetmed.wisc.edu

Experiments involved male Sprague–Dawley rats (n = 8; weight, 285 to 330 g; Harlan Laboratories, Madison, WI). Rats were housed in pairs in polypropylene cages, and a 12:12-h light:dark cycle was maintained for the duration of the study. Commercial diet (Harlan Teklad 7002, Harlan Laboratories) and tap water were provided ad libitum.

Induction of stifle arthritis. For induction of stifle arthritis, rats were anesthetized with an isoflurane–oxygen mixture delivered by facemask. The isoflurane vaporizer initially was set at 4% with an oxygen flow rate of 1 L/min; once relaxation began to occur, the isoflurane vaporizer was reduced to 2% for the remainder of the anesthetic period. The anesthetic plane was determined to be sufficient when the rat did not exhibit a withdrawal reflex to a strong toe pinch and had a regular breathing pattern. Complete Freund adjuvant (CFA) was prepared by thoroughly mixing *Mycobacterium butyricum* (60 mg, Voigt Global Distribution, Lawrence, KS), saline (0.9%, 4 mL), Tween 80 (1 mL), and paraffin oil (6 mL). The mixture was autoclaved (120 °C, 20 min) to rupture the mycobacterial cell walls.[4,6,12,24,28] The solution was refrigerated and stored for less than 1 mo prior to injection. The left stifle was clipped and sterilely prepared with povidone–iodine solution and warm saline. The stifle joint was palpated to locate the femurotibial articulation; a dissecting microscope was not used. Warm and well-mixed CFA (0.05 mL) was injected into the left stifle intraarticularly by using a 26-gauge needle as previously described.[4] Rats were allocated randomly to 3 treatment groups (LE-hydromorphone, standard hydromorphone, and empty liposomes).

Preparation and administration of LE-hydromorphone. Ammonium-sulfate–gradient-loaded hydromorphone liposomes were made according to our previously described method.[15,31] Hydromorphone (40.02 mg; Sigma-Aldrich, St Louis, MO) was substituted for oxymorphone, the lipid composition was dipalmitoylphosphatidylcholine: cholesterol (2:1), and the encapsulation efficiency was determined spectrophotometrically at 282 nm, corrected for lipid content by using the 260-nm absorbance.

LE-hydromorphone liposomes were stored at 4 °C, protected from light, for less than 1 mo. Our laboratory has determined that storage time of less than 1 mo under these conditions results in minimal leakage or deterioration of the LE-hydromorphone preparation. Furthermore, storage of LE-hydromorphone in the dark for more than 6 mo at 4 °C is associated with a 5% to 10% loss of opioid content from the liposome. Therefore, if the LE-hydromorphone is stored appropriately, the concentration should be retained at 90% to 95% of the original value over a 6-mo period. The preparation used in this study was used within 1 mo of quantitation, such that less than 5% loss might have been expected at the time of epidural injection.

Assessment of pain. ***Thermal sensitivity.*** Thermal sensitivity as an indicator of pain threshold was measured by using latency of withdrawal of the left hindlimb from a radiant heat stimulus.[7] Stimulus intensity and rate of heating of the thermistor was kept constant throughout the study to establish an average of a 10-s withdrawal latency in a normal animal during baseline (prearthritis) readings. Maximal time of heat exposure for all measurements was cut off at 20 s to prevent thermal burns. Rats were habituated to the thermal latency device (Ugo Basile, Varese, Italy) for 10 min prior to any measurements. Latencies were calculated as an average of 4 to 6 measurements; 1 or 2 latency measurements were taken in succession. The rat then had a 5- to 10-min rest period before another 1 to 2 latencies were obtained, with another 5- to 10-min rest period before the final 2 measurements. This pattern allowed sufficient time between latency measurements to prevent learned responses or the development of hyperalgesia secondary to repeated noxious stimuli in quick succession.[17] External stimuli (noise, changes in lighting) were minimized. Von Frey measurements for tests of allodynia were not possible due to economic limitations on equipment purchase.

Incapacitance. Incapacitance measures the weight bearing (in grams) of each hindlimb in a chamber where the rats placed their weight almost exclusively on the hindlimbs with only the tail outside the test chamber. Animals were habituated to the incapacitance device (IITC Life Science, Woodland Hills, CA) for 10 min prior to any measurement. Weight bearing (in grams) was calculated as an average of 35 to 40 5-s measurements.

In all groups, baseline measurements of incapacitance and thermal withdrawal latency were established by averaging measurements taken at 3 distinct times, between 0800 to 1000 h on 3 consecutive days, prior to CFA injection. After CFA injection, measurements were taken beginning 24 h after CFA injection to confirm that arthritis-related pain had been induced. Measurements were taken daily for 2 more days to confirm consistency in the reduction of latency values after CFA-induced arthritis. Epidural drugs then were administered, and measurements were taken again at 2, 4, and 6 h and daily afterward for 4 d after drug administration. All measurements, including baseline and after arthritis was induced, were taken at approximately the same time each morning, between 0800 and 1000 h. Thermal withdrawal was used to assess pain threshold after monoarthritis induction with CFA. In all groups, a decrease in thermal withdrawal latency was used to confirm induction of monoarthritis in the left stifle. All rats exhibited a reduction in thermal withdrawal latency after CFA injection.

Subjective behavioral assessments. The degree of lameness induced by monoarthritis and systemic behavioral side effects of epidural administration were assessed by using 2 ethograms (Figures 1 and 2) prior to other testing. To ensure consistent scoring, a single person who was not blinded to treatments performed these subjective assessments throughout the study. Assessments were conducted by observing the animal undisturbed in its cage for 10 to 15 min prior to removing it for threshold tests. The degree of lameness (Figure 1) was assessed by observing how the rat moved about its cage (mobility) and by how much it appeared to weight-bear on the affected limb (stance). Systemic behavioral effects (Figure 2) were assessed by evaluating the Straub phenomenon (tail rigid, progressing to tail extended 90º from body), eye protrusion, and either excessive or obtunded movement.

Epidural injections. The doses of LE-hydromorphone that were chosen for this study were based on a pilot study we conducted in dogs that received 2 different formulations of liposome-encapsulated hydromorphone by the epidural route. The dose used in this study for rats was extrapolated from the dog pilot study by comparing relative dosage recommendations for oxymorphone (twice the potency of hydromorphone) between dogs and rats.[10] The doses of LE-hydromorphone used in the current study were 20 times greater than those for the standard drug formulation because the liposomal membranes of the encapsulated formulation form a reservoir that slowly releases the drug. The dose level for LE-hydromorphone also was based on pharmacokinetic results from experiments in our laboratory on rhesus macaques, in which LE-hydromorphone given systemically was shown to have a very slow release rate and achieve serum concentrations comparable to those of the standard formulation only after dosages 10 to 20 times greater were used.[15]

Score	Mobility	Stance
0	Walks and runs normally	Rat stands bearing weight equally on all 4 limbs
1	Walks and runs with difficulty	Stands, bearing some weight on the arthritic limb
2	Walks with difficulty	Stands with the arthritic paw touching the floor, toes curled under
3	Crawls only	Stands on 3 paws only
4	Lies down only	Not applicable

Figure 1. Subjective behavior assessment scoring system for lameness.

Epidural injections were administered 96 h after induction of monoarthritis with CFA, after a decrease in thermal withdrawal latency had been confirmed for 3 d, between vertebrae L5–L6 or L6–S1 by using a 25-gauge needle. Briefly, an area of skin over the L5–L6 or L6–S1 vertebrae was clipped and prepared in a sterile manner after the anatomic landmarks had been identified by palpation in line with the ileal wings. The needle was advanced through the skin with a drop of sterile saline until a loss of resistance was noted. Tail twitch then was used to determine accurate needle placement in the epidural space. The same person (JRS) performed all epidural injections. All rats were lightly anesthetized with isoflurane in oxygen by mask for the epidural injection, and recovery occurred within 5 min.

To avoid the possibility that rats would become tolerant to the CNS effects of the opioid over time,[18] each rat received only 1 epidural drug administration during the course of the study. Rats were divided randomly into 3 groups prior to epidural injections. One group of rats received 2.0 mg/kg LE-hydromorphone in a volume of 0.3 mL; the second received 0.1 mg/kg standard-formulation hydromorphone HCl (Hospira, Lake Forest, IL) in a volume of 0.3 mL; the remaining rats received 0.3 mL gradient-purified liposomes containing buffer. Injections were completed over 30 s. Behavioral assessments, thermal withdrawal latency testing, and incapacitance testing were conducted in all rats at 2, 4, and 6 h after epidural injections and then daily for 4 d.

Histopathology of the spinal cord. On the final day of the study, 96 h after epidural injections, all rats were anesthetized deeply with isoflurane and euthanized with intracardiac injection of 1.0 mL supersaturated KCl. Samples of spinal cord and vertebral column were removed promptly from euthanized rats and placed in 10% neutral buffered formalin. Samples were embedded and processed for routine hematoxylin and eosin staining. One section each of cervical, thoracic, and lumbar spinal cord was examined by a board-certified veterinary pathologist (RS) blinded to treatment condition.

Statistical analysis. One-way ANOVA with a Tukey HSD posthoc test (version 11, SPSS, Carey, NC) was used to compare behavioral measures between groups and postCFA values with postepidural values for thermal withdrawal latency, incapacitance, and behavioral scoring. A P value of less than 0.05 was considered significant.

Results

Assessment of pain. Behavioral signs of monoarthritis occurred in all rats, as evidenced by increased lameness scores 3 d after CFA injection and by changes in thermal withdrawal latencies. Lameness scores in all 3 groups were significantly ($P < 0.05$) higher after CFA monoarthritis was induced, with scores generally ranging between 1 to 1.5 (data not shown). We did not, however, perform histopathology on arthritic joints to confirm and evaluate the extent of arthritis. Because all 3 groups had consistent behavioral evidence of pain after CFA injection, we postulate that an equivalent arthritic change occurred in all animals but cannot confirm this hypothesis without histopathology.

A significant ($P < 0.001$) decrease in thermal withdrawal latency after CFA injection occurred in all rats (Figure 3). The contralateral paw was not tested as a control because plastic changes occur within the contralateral spinal cord dorsal horn after painful injury[25] and 'cross talk' occurs in models of bone loading and growth between ipsi- and contralateral sides of the spinal cord.[23] We included a control group of rats that received no analgesic in their epidural injections (saline only), and we compared baseline and monoarthritis data from individual rats with the data obtained from the left hindpaw after epidural injection. Rats that received LE-hydromorphone showed significant increases in thermal withdrawal latency at 2 h ($P = 0.0001$), 4 h ($P = 0.0002$), 6 h ($P = 0.0005$), 24 h ($P = 0.02$), 48 h ($P = 0.04$), and 72 h ($P = 0.02$) after epidural injection of the drug (Figure 3). Thermal withdrawal latency in these rats were not statistically different from baseline values at time points through 72 h. Rats given standard hydromorphone had no statistically significant increase in thermal latency at any time point after epidural drug administration (Figure 3). Saline-treated arthritic rats showed no significant change in withdrawal latencies at any time point after the epidural (Figure 1).

Despite a decrease in thermal withdrawal latency after induction of arthritis with CFA, no groups showed a significant decrease in weight bearing on the left hind limb, as measured with the incapacitance meter, compared with baseline ($P < 0.20$; Figure 4). Rats given LE-hydromorphone showed significant increases in weight-bearing on the lame limb at 2 h ($P = 0.01$), 4 h ($P = 0.01$), 6 h ($P = 0.04$), 24 h ($P = 0.04$), and 96 h ($P = 0.03$) after epidural drug administration as compared with postCFA weight-bearing (Figure 4). Control rats and those that received standard hydromorphone showed no increase in left hindlimb weight-bearing as compared with postCFA values at any time point after epidural administration.

Subjective lameness assessments as described in Figure 1 were performed for all 3 groups of rats (Figure 5). Compared with postCFA values, lameness behavior scores were significantly reduced in LE-hydromorphone-treated rats at 2 h ($P = 0.03$), 4 h ($P = 0.002$), 6 h ($P = 0.01$), 24 h ($P = 0.01$), 48 h ($P = 0.01$), and 96 h ($P = 0.03$). In rats given standard hydromorphone, pain scores were significantly lower than postCFA values only at 2 h after epidural administration ($P = 0.03$). There was no change at any time point in pain scores compared with postCFA values in control rats.

Subjective behavioral assessment. Two rats that received LE-hydromorphone showed extreme CNS effects (cumulative score greater than 5), characterized first by constant movement followed by stiffness and nonresponsiveness. These 2 rats died of respiratory failure within 6 h of epidural injection and were excluded from data analysis. These 2 rats were replaced, and other similar occurrences did not occur in this group. Rats given LE-hydromorphone generally exhibited greater CNS effects than did those that received standard hydromorphone at 2 h ($P = 0.01$), 4 h ($P = 0.003$), and 6 h ($P = 0.0006$; Figure 6). Typical behavior changes included an increase in unprovoked movement within the cage, slight stiffness in posture, and a

Score	CNS excitement	Behavior	Straub reflex
0	Normal behavior	Normal	Normal body position
1	Increased movement in cage	Rigid, stiff	Eyes slightly protruded; body stiff; tail extended behind body
2	Constant movement in cage	Obtunded, but moves when prodded	Eyes dramatically protruded; tail rigid and at a 90° angle to body
3	Jumping, startled at noise, vocalizing	Obtunded; does not move when prodded	Not applicable

Figure 2. Subjective behavior assessment scoring system for CNS effects.

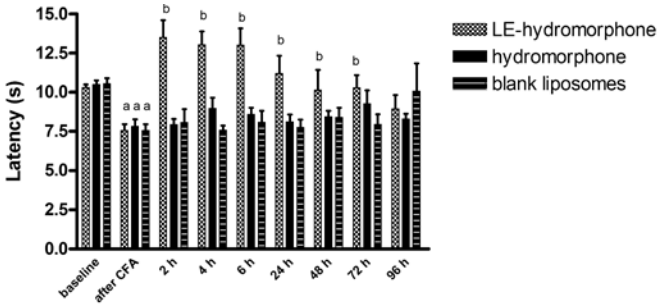

Figure 3. Thermal withdrawal latencies (s; mean ± SE) in rats treated epidurally with LE-hydromorphone (2.0 mg/kg, n = 8), hydromorphone (0.1 mg/kg, n = 8), and blank gradient-purified liposomes (0.1 mL/kg, n = 8). a, significantly ($P < 0.05$) different from baseline value within the group; b, significantly ($P < 0.05$) different from postCFA value within the group.

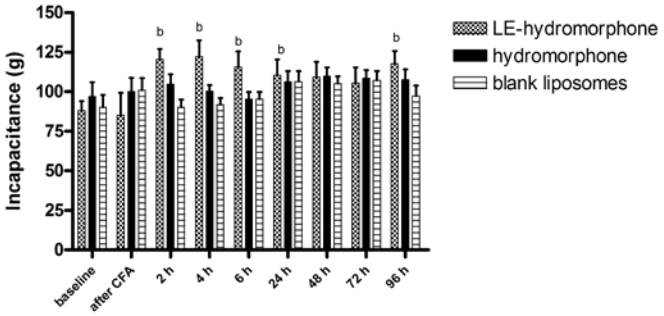

Figure 4. Incapacitance data of left hindlimb (mean ± SE) in rats treated epidurally with LE-hydromorphone (2.0 mg/kg, n = 8), hydromorphone (0.1 mg/kg, n = 8), and blank gradient-purified liposomes (0.1 mL/kg, n = 8). b, significantly ($P < 0.05$) different from postCFA value within the group.

rigid tail with mild eye protrusion. Rats that were considered to be showing CNS effects typically showed an initial increase in motor activity, followed after several minutes by a mildly obtunded stance, eye protrusion and a stiff tail. We monitored the progression in CNS signs in these rats for 10 to 15 min; their initial increase in movement often changed to a decrease in movement (obtunded) after several minutes. According to the behavioral scoring system used, the CNS effects in rats given LE-hydromorphone were still considered mild. No control rats exhibited any changes in CNS behavior. By 24 h after epidural administration, CNS behavior was scored as normal for all rats in all groups.

Histopathology of the spinal cord. Hematoxylin and eosin staining of the spinal cord revealed no grossly obvious pathologic lesions as a result of the epidural injection or the presence of the drug within the epidural space.

Discussion

The purpose of the current study was to assess the duration of LE-hydromorphone administered epidurally in a rat model of monoarthritis. In all rats, monoarthritis was confirmed by increased lameness scores and by the significant decrease in thermal withdrawal latency 24 h after CFA was injected into the stifle joint. Our data indicate that a single dose of epidurally administered LE-hydromorphone can provide hindlimb analgesia for at least 72 h when measured by subjective behavioral assessment and thermal withdrawal latencies.

This study used the monoarthritis rodent model to assess the analgesia obtained with epidural administration of standard hydromorphone or a slow-release formulation, LE-hydromorphone. In this and other models of arthritis, central sensitization can occur,[24] resulting in spinal cord afferent sensory neurons that are more sensitive to mild nociceptive input (thermal hyperalgesia) and with wider receptive fields, leading to referred pain from sites other than that of the arthritis. In chronic pain states in which hyperalgesia occurs, and particularly in neuropathic chronic pain states, opioids tend to be less effective,[5] due to a number of postulated mechanisms that are beyond the scope of this discussion. Therefore, the model we used was more appropriate than a chronic neuropathic pain model such as loose sciatic ligation.[1] However, the development of hyperalgesia of chronic pain may explain why we saw minimal to no analgesia after standard hydromorphone was injected epidurally. The clear analgesia that occurred after LE-hydromorphone injection could have been partially related to the high dose that was administered, given that in chronic hyperalgesia states, opioids can be effective if provided in extremely high doses.[5]

Although CFA induced monoarthritis in all rats, the rats did not show reduced weight-bearing in the affected hindlimb according to incapacitance testing. In this device, rats are positioned statically on the sensors, suggesting the possibility that arthritic pain would not change weight-bearing while animals are immobile. Had we been able to perform dynamic force plating with the rats in movement, we likely would have seen a decrease in weight-bearing at the walk, consistent with observations during lameness scoring. After epidural injection of LE-hydromorphone, affected limbs demonstrated prolonged increases in weight-bearing, according to the incapacitance meter.

Opioid analgesics typically have longer durations of effectiveness when administered epidurally than systemically, due to slow uptake from the spinal cord dorsal horn; however, with standard formulations, this duration is usually still on the order of hours rather than days. For example, epidural hydromorphone (0.2 mg/kg) lasts 6 to 8 h in dogs,[27] whereas epidural morphine sulfate lasts 12 to 24 h.[30] Epidural opioids often are used in clinical medicine to treat hindlimb pain after surgery and are preferable to systemic administration because of their

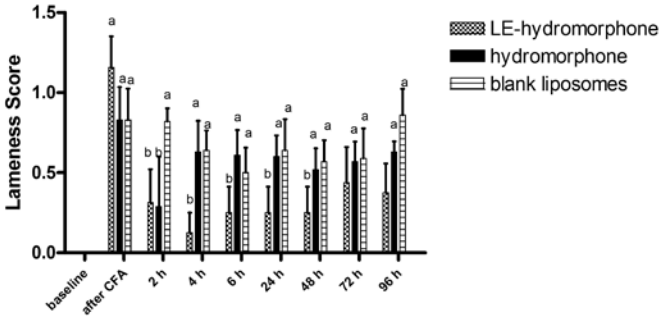

Figure 5. Cumulative subjective lameness behavior assessment (mean ± SE) based on the scoring system described in Figure X in rats treated epidurally with LE- hydromorphone (2.0 mg/kg, n = 8), hydromorphone (0.1 mg/kg, n = 8), and blank gradient-purified liposomes (0.1 mL/kg, n = 8). a, significantly ($P < 0.05$) different from baseline value within the group; b, significantly ($P < 0.05$) different from postCFA value within the group.

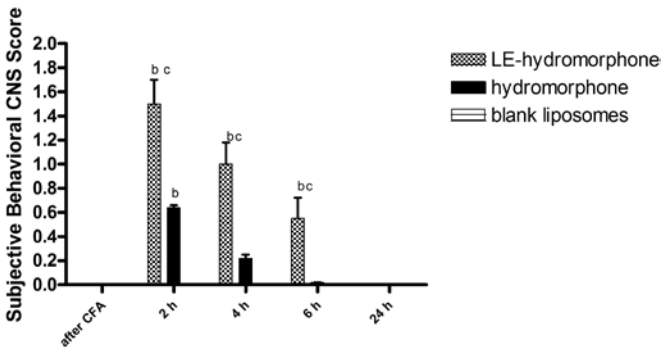

Figure 6. Cumulative subjective CNS behavioral assessment (mean ± SE) based on scoring system described in Figure Y in rats treated epidurally with LE- hydromorphone (2.0 mg/kg, n = 8), hydromorphone (0.1 mg/kg, n = 8), and blank gradient-purified liposomes (0.1 mL/kg, n = 8). b, significantly ($P < 0.05$) different from postCFA value within the group; c, significantly ($P < 0.05$) different from hydromorphone at that time point.

more regional effect and associated reduction in systemic side effects, such as sedation or nausea.

A commercial liposome-encapsulated formulation of morphine (Depodur) has been shown to provide 48 h of analgesia after epidural or intrathecal administration in rats, dogs, and humans.[14,19,33-36] This formulation is approved by the Food and Drug Administration for use in humans. Because the medication is provided as a single epidural injection, infusion pumps, indwelling epidural catheters, and patient-controlled analgesia are not required, thus obviating potential problems with epidural catheter dislodgement, disconnection, or contamination. A safety concern for Depodur is the potential for long-lasting respiratory depression or sedation, particularly in elderly or debilitated patients.[32] In a randomized multicenter trial,[8] patients that received 1 of 3 dose levels of Depodur for pain after knee arthroplasty required a 3-fold lower amount of postoperative opioid doses, with some patients requiring no additional opioid analgesia. Adverse events included nausea (78%), pyrexia (46%), vomiting (43%), pruritis (43%) and hypotension (36%), and 4 patients (all of whom were older than 65 y) among the 168 experienced serious respiratory depression.[8] Some veterinary species, including dogs, are less likely to manifest the more commonly recognized side effects of epidural opioid administration,[30] but whether rodents can be included in this category is unknown.

In the current study, we observed transient CNS excitement in rats given LE-hydromorphone compared with standard hydromorphone after epidural administration. This CNS excitement was generally minimal and reflected an increase in stiffness. The observer scoring subjective behavior and lameness was not blinded to treatment, so a potential bias exists in these data. Both the lameness and behavioral scoring systems were numerical, meaning that a given observed behavior was assigned a single numerical value, rather than a subjective score (for example, as is done with visual analog scaling in pain assessment). However, our approach is likely to have assessed the incidence of CNS excitement accurately in rats given LE-hydromorphone. In animal models, opioid-induced neuroexcitation is reversible with naloxone, suggesting that this phenomenon is a direct effect of agonist opioid drugs.[22] Opioid-induced effects on excitatory or inhibitory neurotransmitters may play a role also. N-methyl-D-aspartate receptor-mediated excitatory glutamate responses have been suggested as a mechanism.[3,11]

Two rats that received LE-hydromorphone developed extreme CNS excitement and died, suggesting that hydromorphone was rapidly available after injection in these 2 animals. The reason for this effect in these 2 rats is unclear, although our studies suggest that hydromorphone is released from liposomes more rapidly after epidural injection in rats than occurs after injection by other routes in other species. The shear forces required for rupture of the liposome membranes that were used in this study (dipalmitoyl–phosphatidyl choline and cholesterol) make it physically unlikely that fast injection through a 26-gauge needle would result in lysis of the liposomes. Studies in dogs[29] have shown that the liposome preparation contains a small proportion of free drug and that liposomes leak more rapidly early after administration than at later time points. The small size of the rat epidural space and the ease with which LE-hydromorphone could be injected intrathecally and travel the relatively short distance to the higher brainstem and brain may account for the cases of extreme CNS excitement followed by respiratory failure. Furthermore, these 2 fatalities occurred early during the study, when the technique of the operator had not been developed fully, given that other incidents of possible intrathecal injection did not occur with subsequent subjects. The possibility of intrathecal injection of drugs in the current study could have been reduced by surgical preplacement of epidural catheters and contrast confirmation of placement.[14]

LE-hydromorphone did not induce any observable histologic changes to the spinal cord or meninges, based on hematoxylin and eosin staining. The evaluation did not test for the presence of reactive astrocytes as a result of the peripheral inflammation of the monoarthritis model, nor did it measure cytokines that are upregulated within the dorsal horn in the presence of chronic pain states.[2,21]Aseptic technique was used for all epidural injections.

In the current study, LE-hydromorphone provided prolonged relief from pain associated with stifle monoarthritis, with superior duration when compared with epidurally administered standard hydromorphone. Because we did not compare multiple doses of standard hydromorphone with LE-hydromorphone and because we did not observe appreciable analgesia with our single dose of standard hydromorphone, this study did not fully evaluate the efficacy of LE-hydromorphone but rather simply documented extended duration of analgesia. The extreme CNS excitement and death from respiratory failure in 2 of 10 rats that received LE-hydromorphone warrants caution when injecting liposome-encapsulated opioid preparations. The extended duration of action of epidural LE-hydromorphone

reported in the current study exceeds, to our knowledge, that of any currently available extended release opioid formulation. Therefore, further study into the safety and practicality of using LE-hydromorphone is warranted in light of the important implications of this preparation for animals requiring prolonged hindlimb analgesia.

Acknowledgments

This work was supported by the NIH National Center for Research Resources (grant 2R01-RR018802) and the Merck–Merial Summer Scholars Program. We thank Brynn K Schmidt for technical assistance.

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