Serum concentrations and analgesic effects of liposome-encapsulated and standard butorphanol tartrate in parrots

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Objective—To compare serum concentrations of liposome-encapsulated butorphanol tartrate (LEBT) and standard butorphanol tartrate (STDBT) following SC and IM administration, respectively, and to evaluate analgesic effects of LEBT and STDBT after parenteral administration to Hispaniolan parrots.

Animals—11 adult Hispaniolan parrots.

Procedure—The ability of LEBT to prolong the duration of analgesia in an avian species was tested. Blood samples were collected at serial time points after SC administration of LEBT (10 mg/kg or 15 mg/kg) or IM administration of STDBT (5 mg/kg). Serum concentrations of butorphanol tartrate were determined by use of a commercial immunoassay that measured parent drug and metabolites. Analgesic efficacy was evaluated in parrots exposed to electrical and thermal stimuli. Foot withdrawal thresholds were recorded at baseline and at serial time points after LEBT (15 mg/kg), liposome vehicle, STDBT (2 mg/kg), or physiologic saline (0.9% NaCl) solution administration.

Results—LEBT had a prolonged in vivo release for up to 5 days. Negligible serum butorphanol and butorphanol metabolite concentrations were obtained at 24 hours after IM administration of STDBT. Analgesic efficacy of LEBT as measured by foot withdrawal threshold to noxious thermal and electrical stimuli persisted for 3 to 5 days following SC administration of LEBT.

Conclusions and Clinical Relevance—SC administration of LEBT provided analgesia and detectable serum butorphanol concentrations in Hispaniolan parrots for up to 5 days. The use of LEBT may allow for substantial improvement in long-term pain relief without subjecting birds to the stress of handling and multiple daily injections. (*Am J Vet Res* 2006;67:775–781)

Abbreviations

LEBT	Liposome-encapsulated butorphanol tartrate
STDBT	Standard butorphanol tartrate
LV	Liposome vehicle

Understanding and treating painful conditions in animals have gained considerable and deserved attention in recent years. It is now standard practice to provide pain relief to animals under conditions considered painful in humans, and the Institute of Laboratory Animal Resources Guide mandates pain relief for all vertebrate animals.[1] However, effective treatment of animal pain can be problematic because of distress associated with frequent handling for parenteral drug administration and the short duration of drug efficacy, especially in small animals with high metabolic rates. Additionally, evaluating analgesic efficacy in animals is challenging because objective measures of behavior associated with pain are difficult to define in nonverbal species.[2-5] This is particularly true in less traditional animal species, such as birds, small mammals, reptiles, amphibians, and fish. Many bird species are common companion and laboratory animals, and an urgent need exists to understand and manage pain in avian species.

Several recent advances in avian analgesia have supported the clinical application of analgesic drugs for the avian patient.[6-17] Previous studies[7,11,16] have validated the clinical use of opioids for birds, particularly those with κ-opioid receptor affinities. Opioids are the most effective class of analgesic drugs for perioperative pain. Butorphanol tartrate, a mixed opioid agonist-antagonist with μ-opioid receptor antagonism and κ-opioid receptor agonism, is currently considered the analgesic drug of choice for acute and chronic pain management in birds.[7,11,15] However, an accepted dose of butorphanol tartrate (2 to 4 mg/kg) in psittacine birds has a short plasma half-life and requires redosing every 2 to 4 hours.[15] Long-acting opioid drugs would address the issue of frequent dosing. In humans and other mammals, long-acting opioids are associated with fewer adverse effects and have a lower abuse potential because of the absence of the bolus effect frequently associated with standard opioid preparations.[18-26] Encapsulation of opioids into lipsomes is 1 method for producing a sustained-release preparation. Liposome-encapsulated morphine produces substantial plasma concentrations of parent drug and metabolites for 6 days after a single SC injection in mice.[26] Liposome-encapsulated morphine and oxymorphone provided effective analgesia in rats with induced neuropathic pain for 7 days following a single SC injection.[24] However, long-acting, liposome-encapsulated opioids currently designed for humans and laboratory mammals may be less effective in avian species because

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morphine and other μ-receptor opioid agonists have little efficacy in psittacine birds commonly kept as companion animals.[11,15,16]

Understanding normal behavior in a particular species and the ability to quantitate abnormal behaviors indicative of pain are critical to the study of analgesia.[2-5] In addition, the context in which the behavior is being observed influences the expression of behavior.[4,5] The use of a novel perch that can deliver thermal and electrical noxious stimuli to the plantar surface of a bird's foot has been described previously[27] and is an objective behavioral method for assessing pain and analgesia. This device can be used to evaluate the effects of different analgesic drugs on perceived discomfort by measuring foot withdrawal thresholds to increasing intensities of thermal and electrical stimuli and comparing these changes to baseline values. This perch design provides an ecologically relevant and objectively defined behavioral analgesimetry method for use in avian species. This technique is a sensitive, reliable, and valid method across species and individuals and is readily quantifiable.[27,28] Objectives of the study reported here were to formulate LEBT, compare the serum concentrations of analgesics in Hispaniolan parrots following STDBT (5 mg/kg, IM) and LEBT (10 and 15 mg/kg, SC) administration, and compare the analgesic efficacy of LEBT (15 mg/kg, SC), LV, STDBT (2 mg/kg, IM), and physiologic saline (0.9% NaCl) solution in Hispaniolan parrots by use of thermal and electrical noxious stimuli.

Materials and Methods

Animals—Eleven adult Hispaniolan parrots (*Amazona ventralis*) of unknown sex weighing 271.25 ± 10.8 g (mean ± SD) were used in this study. All parrots have been in the teaching and research flock for > 10 years and were healthy prior to and during the study. During the nonstudy period, parrots were housed as a social group in a 3.7 × 3.7-m room equipped with artificial tree branches and bird-appropriate toys for environmental enrichment. During the study, parrots were housed individually in standard, stainless steel laboratory cages (0.6 × 0.6 × 0.6 m). They were maintained on a 12-hour light-to-dark cycle, fed a commercial pelleted diet for psittacine birds,[a] and provided water ad libitum. An Institutional Animal Care and Use Committee at the University of Wisconsin School of Veterinary Medicine approved the experimental protocol.

Study design—A within-subjects, repeated-measures experimental design was used to evaluate serum concentrations of butorphanol tartrate and its metabolites following LEBT and STDBT[b] administration. Parrots were randomly assigned to 2 LEBT groups as follows: 8 parrots received LEBT at 15 mg/kg, SC, and 4 parrots received LEBT at 10 mg/kg, SC. Two randomly selected parrots were included in both groups, receiving LEBT at 15 mg/kg first, followed by a washout period of > 90 days prior to receiving LEBT at 10 mg/kg. Approximately 1 year prior to LEBT administration, 4 parrots were randomly assigned to receive STDBT at 5 mg/kg, IM.

A within-subjects, complete crossover experimental design was used for analgesimetry experiments. Parrots (n = 11) received LEBT (15 mg/kg, SC) and were tested in the analgesimetry apparatus. After a washout period of 22 or 24 days, the same parrots received LV (administered at equal volume of LEBT, SC) and were tested again in the apparatus. The same parrots (n = 11) were randomly assigned to receive STDBT (2 mg/kg, IM) or saline solution (administered IM at an equal volume as STDBT) after a period of 42 or 48 days following LEBT administration. After a washout period of 13 or 24 days, these same parrots were crossed over to the alternate treatment.

Preparation of LEBT—Liposomes containing butorphanol tartrate were prepared by use of dehydration-rehydration method as described previously for oxymorphone.[23,24,29] Powdered butorphanol tartrate[c] (59.64 mg) was dissolved in 1mM sodium citrate buffer (pH, 4.0) to form a 12.5mM solution of butorphanol. The solution was sterilized by use of a 0.22-μm-pore diameter filter.[d] A film containing 80 μmol of egg phosphatidylcholine was dried onto the walls of an 18 × 160-mm screw-capped culture tube by use of a rotary evaporator; the water bath temperature was set at 37°C. The dried film of egg phosphatidylcholine was overlaid with the butorphanol solution. The mixture was sonicated in a cylindrical bath[e] for 10 minutes; transferred to sterile, round-bottomed flasks; frozen in a dry ice and isopropanol bath; and lyophilized for 24 to 48 hours. The lyophilized mixture was rehydrated for 30 minutes with 0.5 mL of sterile, distilled water for irrigation to form liposomes. After hydration, the liposomes were diluted with 9.5 mL of sterile physiologic saline solution and 10mM acetate buffer (saline

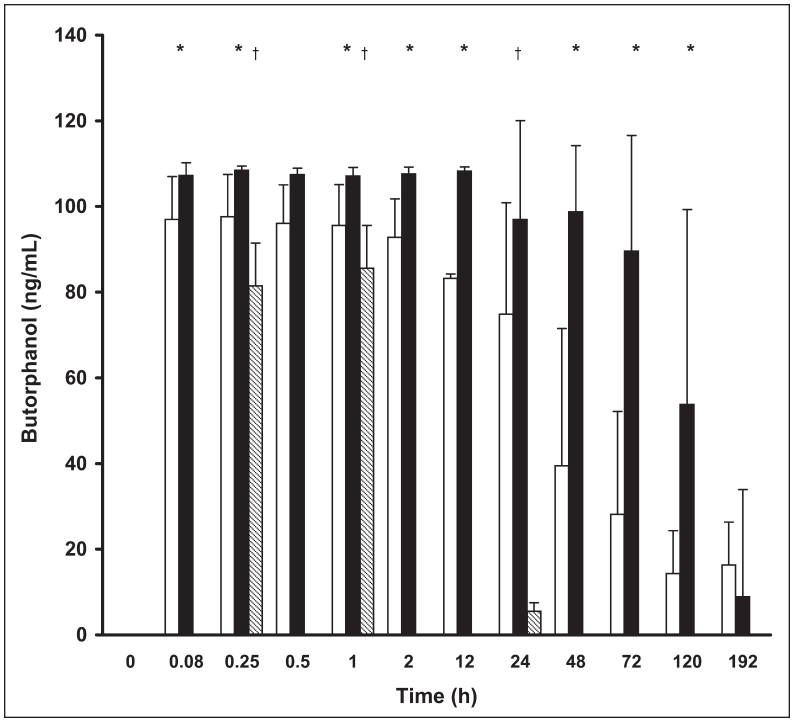

Figure 1—Mean ± SD total serum butorphanol (and butorphanol metabolite) concentrations in Hispaniolan parrots after SC administration of LEBT at 10 mg/kg (n = 4; open bars) or 15 mg/kg (8; closed bars) and after IM administration of STDBT at 5 mg/kg (4; striped bars). Blood samples were collected at baseline and 0.08, 0.25, 0.5, 1, 2, 12, 24, 48, 72, 96, 120, and 196 hours after LEBT administration and at 0.25, 1, 24, and 48 hours after STDBT administration. *Significant ($P < 0.05$) differences between LEBT (15 mg/kg and 10 mg/kg) groups. †Significant ($P < 0.05$) differences between LEBT (15 mg/kg) and STDBT groups.

solution-acetate buffer [pH, 4.0]). Liposomes were centrifuged for 10 minutes at 1,000 × *g*, and the excess buffer containing unencapsulated drug was removed by aspiration. The resulting pellet was resuspended in 9.5 mL of fresh saline solution-acetate buffer, then was sedimented twice more as described. After the third sedimentation, liposomes were resuspended in 2 mL of fresh saline solution-acetate buffer and stored at 5°C. Liposome preparations were analyzed for their butorphanol content after solubilizing 200 µL of the liposome preparation in a solvent solution containing 600 µL of methanol and 200 µL of chloroform and agitating the solution gently on a test tube vortex. The solution was placed in a cuvette, and the concentration was determined by its absorbance with the use of a millimolar extinction coefficient of 1.437 cm^{-1} at an absorbance of 281 nm. Saline solution-acetate buffer suspended in the same solvent solution was used as a blank. Liposomes containing saline solution (ie, LV) were used as negative controls for in vivo experiments for comparison.

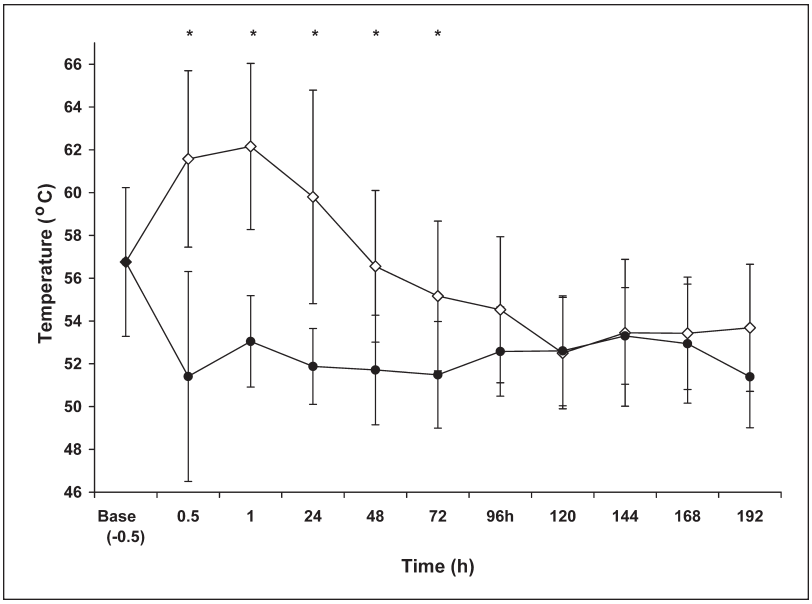

Figure 2—Mean ± SD temperature of foot withdrawal threshold to a noxious thermal stimulus over an 8-day period in Hispaniolan parrots after SC administration of LEBT at 15 mg/kg (n = 11; open diamonds) and after SC administration of LV (11; closed circles). Base = Baseline at 30 minutes prior to drug administration. *Significant ($P < 0.05$) difference between LEBT and LV groups.

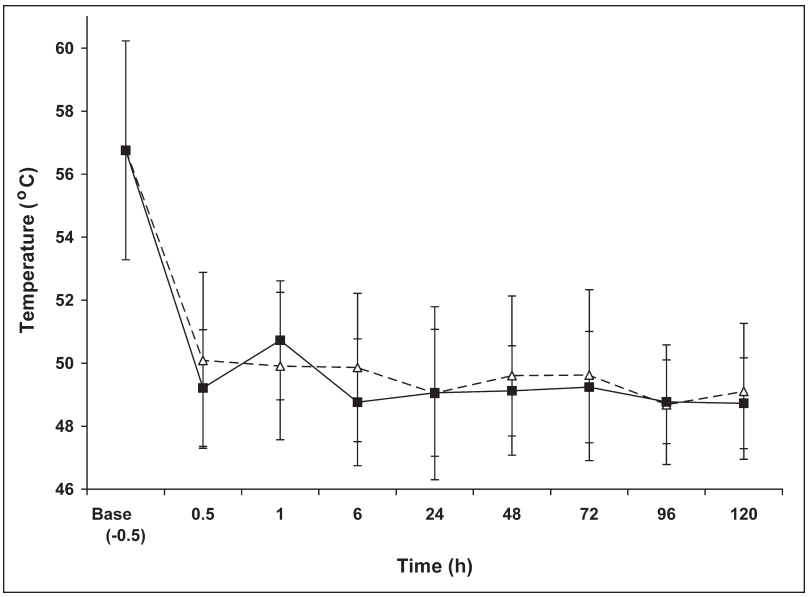

Figure 3—Mean ± SD temperature of foot withdrawal threshold to a noxious thermal stimulus over a 5-day period in Hispaniolan parrots after IM administration of STDBT at 2 mg/kg (n = 11; open triangles) or sterile physiologic saline (0.9% NaCl) solution at an equivalent volume (11; closed squares). Base = Baseline at 30 minutes prior to drug administration.

In vitro release kinetics of LEBT—Cellulose dialysis tubing was prepared by boiling it in EDTA solution and storing it in sterile physiologic saline solution, as previously described.[23,24] Sections of dialysis tubing were filled with either 0.319 mL of serum from a clinically normal Hispaniolan parrot and 0.181 mL of butorphanol liposomes suspended in saline solution-acetate buffer or an equal volume of saline solution-acetate buffer alone. The end of the dialysis tubing was tied, and the bag was placed in 9.5 mL of sterile saline solution for irrigation in a 50-mL conical centrifuge tube. Tubes were prepared in duplicate and placed on a rotary agitator at 20°C. At 3 hours, then daily for 7 days, an aliquot of buffer was removed and placed in a cuvette, and the absorbance at 281nM was determined by use of a spectrophotometer. Each day, the dialysis bag was transferred to a fresh tube containing 9.5 mL of saline solution and replaced on the agitator.[30]

In vivo serum concentrations of LEBT and STDBT—Parrots received a single dose of LEBT (10 mg/kg, SC [n = 4], or 15 mg/kg, SC [8]) or STDBT (5 mg/kg, IM [4]); blood samples (approx 0.2 to 0.3 mL/sample) were collected from the right jugular vein at serial time points to assess the duration of detectable concentrations of butorphanol and its metabolites. The administration of LEBT was by the SC route to form a slow-release drug depot. Doses of LEBT were determined by pilot studies and are commonly administered at 5 to 10 times the standard drug dose because of their slower and prolonged release into circulation.[23-25] The dose of STDBT (5 mg/kg) was chosen just above the high end of the recommended dose range (2 to 4 mg/kg) for psittacine birds[14] on the basis of 2 factors: the variability in analgesic effects between avian species and the goal of trying to ensure that serum concentrations would be detectable for comparison with LEBT. The STDBT was administered IM because this is the recommended route of administration in clinical avian medicine. After drug administration, each parrot was manually restrained and blood samples were collected from the right jugular vein before and at 0.08, 0.25, 0.5, 1, 2, 12, 24, 48, 72, 120, and 192 hours after administration of LEBT. Blood samples were collected from parrots treated with STDBT at 0.25, 1, 24, and 48 hours after injection. Blood samples were collected from a subset of parrots (n = 4) to determine background opioid or other endogenous chemical concentrations that may cross-react by use of an ELISA method.[f]

Analgesimetry behavioral measurements—Thermal and electrical foot withdrawal threshold measurements were collect-

ed on all parrots by use of a behavioral test box equipped with a test perch. The test perch was designed to deliver either an electrical or thermal stimulus to the right or left plantar surface of the parrot's foot.[27] A range of thermal stimuli could be delivered only to the left half of the perch; electrical stimuli could be delivered at increasing amperage only to the right half of the perch. The analgesimetry test box had dark sides that inhibited the parrot from viewing its surroundings, including the observer, and a clear front that allowed the observer to monitor behavioral responses by use of a remote video camera. Prior to the experiment, each parrot was acclimated to the test chamber for a minimum of 30 min/d, for 7 days.

An electrical stimulus (range, 0 to 1.4 mA) was delivered through the aluminum perch to an electrode applied to the parrot's right metatarsus for 0.5 seconds and repeated at intervals of 20 to 30 seconds. The initial stimulus of 0.07 mA was increased by 5% increments until a withdrawal response was observed. An electrical threshold withdrawal response was defined as 3 consecutive foot withdrawal responses at the same electrical amplitude. The thermal stimulus, generated by thermoelectric modules, ranged from 37° to 69°C and produced a rapid rise and decline in perch temperature. A thermal threshold withdrawal response was defined as the mean perch temperatures of 3 consecutive foot withdrawal responses. A video camera was used to record behavior while the parrot was in the analgesimetry box to prevent the parrot from reacting to the human observer. Baseline thermal and electrical withdrawal threshold data were generated for each parrot on the day prior to analgesic drug or saline solution administration. On day 1 of the experiment, each parrot received LEBT at 15 mg/kg, SC (n = 11). This dose was selected on the basis of serum concentration data from the previous experiment. Analgesimitry testing was performed at 0.5, 1, and 6 hours and daily for a period of 8 days following LEBT administration. After a washout period of 22 to 24 days, each parrot (n = 11) received an SC injection of the control, saline solution–containing liposomes (LV) so that each parrot served as its own control. Serum butorphanol and butorphanol metabolite concentrations returned to nearly baseline concentrations by 8 days after administration of LEBT, and withdrawal thresholds for analgesimetry had returned to baseline at 3 to 5 days, so a washout period of at least 22 days was considered adequate. The same parrots (n = 11) were randomly assigned to receive STDBT (2 mg/kg, IM) or saline solution (administered IM at an equal volume as STDBT) after a 42- to 48-day period following LEBT administration. All parrots were crossed over to the alternate treatment group. The behavioral observer was masked to the treatment conditions when STDBT and saline solution were compared.

Statistical analysis—Serum concentration and analgesimetry data are expressed as mean ± SD values. Sample data distributions were analyzed by use of the Kolmogorov-Smirnov test. In vivo ELISA data were analyzed by use of a Wilcoxon signed rank test statistic[8] to evaluate pairwise comparisons, as the data were not normally distributed.[31] Behavioral analgesimetry data were normally distributed and were analyzed by use of a repeated-measures ANOVA to detect differences between treatment groups. Differences with a value of $P < 0.05$ were considered significant.

Results

Liposome-encapsulation of butorphanol tartrate—Butorphanol tartrate was efficiently encapsulated into liposomes by use of dehydration-rehydration vesicles. Butorphanol tartrate encapsulation efficiency was repeatable within a range of 15.6% to 33% (mean, 22.84%; n = 5 separate batches), resulting in a solution containing 2.2 to 5.26 mg of butorphanol tartrate/mL in 10mM sodium acetate buffer.

In vitro release of butorphanol tartrate—The in vitro release kinetics of LEBT were measured by diffusing preparations of LEBT and parrot serum through dialysis tubing and into a buffer solution for a period of 6 days. After 24 hours, 90% of the butorphanol was released, and after 5 days, 98% of butorphanol had been released into the buffer.

In vivo serum concentrations of LEBT—Background serum concentration of butorphanol, butorphanol metabolites, and other cross-reacting endogenous chemicals was 3.1 ± 0.21 ng/mL, and the lower limit of assay quantification for parrot serum was 1.79 ng/mL. Serum concentrations of butorphanol and butorphanol metabolites were detectable 5 minutes after LEBT administration and persisted at low concentrations on day 8 following SC administration of a single dose (**Figure 1**). Mean serum concentrations of STDBT (5 mg/kg, IM) were greatest when measured 1 hour after injection and were negligible at 24 hours. Daily observations of the parrots led to the subjective conclusion that no overt adverse effects occurred in any parrot that received LEBT, although mild-to-moderate sedation was observed between 1 and 2 hours after injection in some parrots.

Analgesimetry by use of thermal and electrical noxious stimuli—All parrots receiving LEBT had significantly increased thermal foot withdrawal thresholds for 3 consecutive days, compared with control parrots that received LV at similar time points (**Figure 2**).

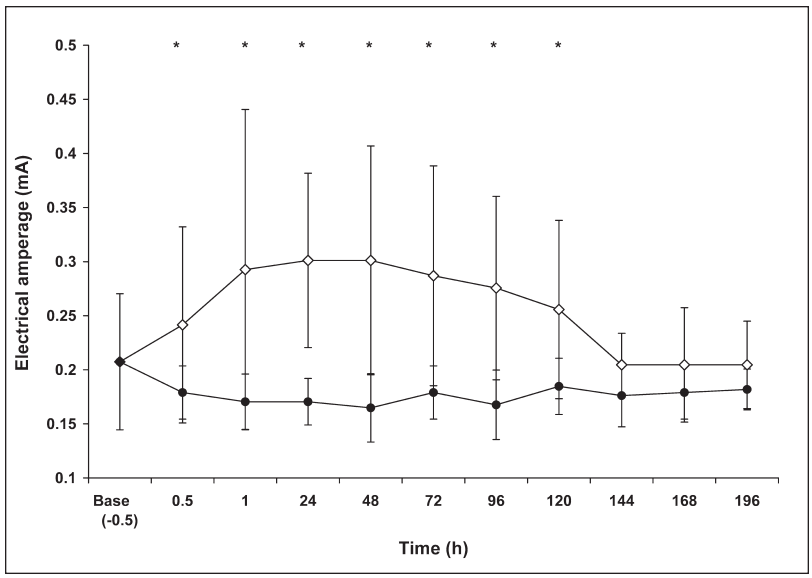

Figure 4—Mean ± SD electrical amperage of foot withdrawal threshold to a noxious electrical stimulus over an 8-day period in Hispaniolan parrots after SC administration of LEBT at 15 mg/kg (n = 11; open diamonds) and after SC administration of LV (11; closed circles). Base = Baseline at 30 minutes prior to drug administration. *Significant ($P < 0.05$) difference between LEBT and LV groups.

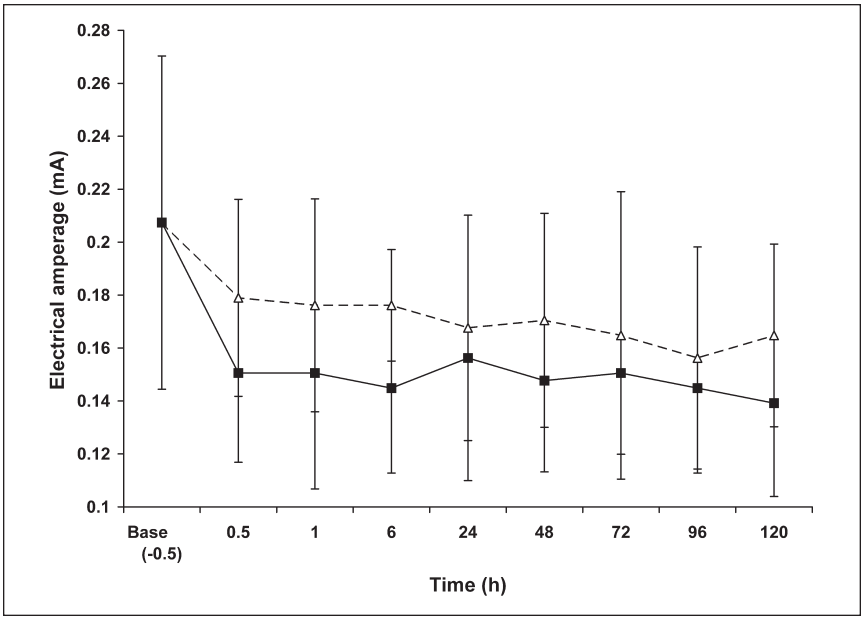

Figure 5—Mean ± SD electrical amperage of foot withdrawal threshold to a noxious electrical stimulus over a 5-day period in Hispaniolan parrots after IM administration of STDBT at 2 mg/kg (n = 11; closed squares) or sterile physiologic saline solution at an equivalent volume (11; open triangles). Base = Baseline at 30 minutes prior to drug administration.

Parrots that received LEBT had mean increased foot withdrawal thresholds to thermal stimuli of approximately 4°C above baseline at 0.5 hours after LEBT administration, 5°C above baseline at 1 hour, and 3°C above baseline at 24 hours, with a return to baseline at 48 hours. In contrast, parrots that received LV had decreased foot withdrawal thresholds, compared with baseline, at all time points. Parrots that received STDBT (2 mg/kg, IM) had foot withdrawal thresholds that were not significantly different from control parrots receiving saline solution (**Figure 3**). Parrots that received a single dose of LEBT (15 mg/kg, SC) had significantly increased foot withdrawal thresholds when exposed to an electrical stimulus for 6 days, compared with control parrots receiving LV (**Figure 4**). Parrots that received LEBT had increased foot withdrawal thresholds to electrical stimuli of approximately 0.04 mA above baseline at 0.5 hours after LEBT administration, 0.08 mA above baseline at 1 hour, and 0.09 mA above baseline at 24 hours and remained elevated above baseline until day 6. In contrast, parrots that received LV had decreased foot withdrawal thresholds, compared with baseline, at all time points. Similar to the thresholds obtained with LV, parrots receiving STDBT (2 mg/kg, IM) or saline solution had thresholds that decreased, compared with baseline (**Figure 5**).

Discussion

Regardless of dose, LEBT provided a long-acting analgesic formulation for use in parrots that was safe and effective for up to 5 days after administration. The extended-release characteristics of the preparation were quantifiable in vitro and in vivo. Our results are consistent with data from mammalian species in which liposome-encapsulated opioids provided safe and effective analgesia in rodents with induced neuropathic and postoperative visceral pain.[19-26] No observable adverse effects were associated with the administration of LEBT in parrots during our study, although mild-to-moderate sedation was observed between 1 and 2 hours after LEBT administration.

Butorphanol tartrate, hydromorphone, and oxymorphone have been successfully encapsulated into liposomes by use of the dehydration-rehydration method.[23-25,29] This method produced controlled drug release over several days in rodents and dogs.[22-26] Butorphanol tartrate was chosen because of its analgesic efficacy in avian species.[11] Unlike morphine sulfate and oxymorphone, which are μ-opioid receptor agonists, butorphanol tartrate is a mixed-opioid agonist-antagonist with κ-opioid receptor agonist and μ-opioid receptor antagonist activities. Current evidence suggests that κ-opioid receptors are quantitatively and qualitatively more important in some avian species, especially psittacine birds.[13,15]

Encapsulation efficiency for butorphanol tartrate was sufficient to produce therapeutically useful concentrations of drug. The in vitro release assay indicated that after 5 days, 98% of the butorphanol was released from the liposomes.

Results of our study indicate that after SC administration of LEBT (10 or 15 mg/kg) in parrots, serum concentrations of butorphanol and its metabolites are measurable for up to 8 days. Serum concentrations were consistently and significantly higher in parrots receiving LEBT at 15 mg/kg, compared with those receiving LEBT at 10 mg/kg, at most time points except day 8; it is not known whether this difference is physiologically relevant because serum concentrations were low for both doses. Serum butorphanol and butorphanol metabolite concentrations were below detection at 24 hours after IM administration of STDBT and were significantly lower than after either dose of LEBT at every time point. These results corroborate those of other in vivo pharmacokinetic studies[19-26] on the use of liposomal opioids in mammals. However, because the assay measured butorphanol and its metabolites, which may not be active analgesics, a direct relationship between serum drug concentrations and pharmacologic efficacy must be inferred. The ELISA was chosen for our study because of the relatively small size of the birds and the number of blood samples required from each bird over time. This assay requires a small quantity of serum and was validated by our laboratory by use of standard butorphanol tartrate in the same species. The parrots in our study had higher serum baseline butophanol and metabolite concentrations, compared with similar ELISAs for measurement of oxymorphone in blood samples of dogs and rats.[24,25] The serum used to measure zero-time background concentrations of butorphanol and butorphanol metabo-

lites was from either butorphanol-naïve birds or birds that had not received any butorphanol for > 90 days; no difference was found between background serum butorphanol and butorphanol metabolite concentrations in birds that have never received butorphanol tartrate and those that may have received it more than 3 months prior. Because of the background concentrations in parrots, our data overestimated the actual serum butorphanol concentrations. A more sensitive assay (eg, high-performance liquid chromatography) may have measured serum concentrations of only butorphanol more accurately in this species of bird. However, our study was not intended to evaluate specific pharmacokinetic parameters but was designed to compare serum butorphanol and butorphanol metabolite concentrations between standard and liposome-encapsulated preparations.

Behavioral data in our study support the relationship between sustained serum concentrations of butorphanol and butorphanol metabolites and clinical analgesic efficacy as measured by foot withdrawal thresholds to 2 noxious stimuli. Increased foot withdrawal thresholds to thermal and electrical stimuli were consistently observed in parrots receiving LEBT, compared with parrots receiving saline solution, LV, or STDBT, and these increased thresholds persisted for 3 to 5 days after SC administration of LEBT. Administration of LEBT provided sustained analgesic effects that were manifested as an increased threshold to noxious stimuli above individual baseline values. This effect was sustained for a mean time of 3 days, when parrots were exposed to the thermal stimuli, and a mean time of 5 days, when exposed to the electrical stimuli. This difference in perception of electrical versus thermal noxious stimuli may be a function of the biological salience of the stimuli to the birds. Under natural conditions, birds would not readily encounter noxious electrical stimuli but could easily come into tactile contact with excessive heat. This could account for a greater tolerance for the novel electrical stimulus and, conversely, less tolerance for the thermal stimulus. An alternative explanation is that no anatomic and physiologic differences exist in pain receptors, nerve fibers, or both in response to thermal and electrical stimuli in birds. Unfortunately, no published data were found on evaluating structural and functional differences of thermal and electrical nociception in avian species. In contrast to LEBT data, foot withdrawal thresholds to thermal and electrical noxious stimuli decreased from baseline in all parrots receiving saline solution, LV, or STDBT. This decrease from baseline in foot withdrawal thresholds may be a function of limited tolerance for the thermal and electrical stimuli in the control groups (saline solution and LV) or an effect of temporal behavioral methods and rapid drug elimination for STDBT. Administration of STDBT at 2 mg/kg, IM, has provided analgesia in other parrot species for a short duration[13,15]; the temporal scale used in our study may not be sensitive enough to detect an analgesic effect in Hispaniolan parrots. An alternative explanation is that butorphanol and any active metabolites are rapidly eliminated from circulation in Hispaniolan parrots; therefore, our behavioral observations were less sensitive for detection of increased foot withdrawal thresholds immediately after administration. An additional explanation is that an interspecific variation exists in analgesic response to butorphanol. In African grey parrots, butorphanol tartrate administered at 1 mg/kg was shown to be isoflurane-sparing, but use of the same dose in Amazon parrots had less of an isoflurane-sparing effect.[6] We selected a dose of 2 mg/kg for our study on the basis of results of previous studies on Amazon parrots[6] and African grey parrots[11]; we expected a change in foot withdrawal threshold at 30 minutes after butorphanol administration. However, because no change in foot withdrawal threshold was found, it is possible that a higher dose of butorphanol is necessary for analgesia in Hispaniolan parrots. The persistent decrease in foot withdrawal thresholds observed in control parrots and in parrots receiving STDBT could have been a function of a conditioned response to repeated stimuli and was not observed in the parrots receiving LEBT because of the analgesic effects.

The stimulus apparatus used for behavioral analgesimetry in animals must be ecologically relevant to the target species, and the technique must have clinical validity, reliability, sensitivity, and quantifiablility.[28] In our study, an aluminum perch was used to deliver a thermal stimulus on the left side and an electrical stimulus on the right.[27] This method has been reliably quantified in cockatoos and African grey parrots.[11,16] The perch design was patterned after the plantar foot withdrawal apparatus used in rodents, in which either thermal or electrical stimuli can be applied to the plantar surface of a rodent's foot. However, the perch design made the apparatus ideal for use in avian species, which fulfilled the ecologic relevance of the apparatus. This method allowed the parrot to freely escape the noxious stimulus by withdrawing its limb from the surface of the perch. Because the thermal or electrical stimulus decays instantly after limb withdrawal, the bird typically placed its foot back on the perch.

Our ability to evaluate, categorize, and quantify painful conditions in animals, especially nondomestic species, is hampered by our limited ability to clearly define pain. The limited number and types of analgesics available for use in these species similarly impair clinical pain management. The behavioral approaches used in our study required species-specific behaviors relevant for the context in which the behavior was expressed and observed. In our study, a single dose of LEBT produced sustained elevations in limb withdrawal thresholds to noxious stimuli, indicating that LEBT provided pain relief for 3 to 5 days in Hispaniolan parrots. These changes were consistent with our findings of sustained serum concentrations of butorphanol and its metabolites. The SC injected LEBT had no clinically relevant adverse effects. The use of LEBT may allow for substantial improvement in the provision of long-term pain relief to birds without subjecting them to the stress of handling and multiple daily injections.

a. Kaytee Exact Rainbow Parrot, Chilton, Wis.
b. Torbugesic-SA (Butorphanol tartrate), Fort Dodge Animal Health, Fort Dodge, Iowa.
c. Butorphanol tartrate salt (99%), Sigma-Aldrich Inc, St Louis, Mo.
d. Gelman Filters, Pall Corp, Ann Arbor, Mich.

e. Laboratory Supplies Co, Hicksville, NY.
f. Neogen, Inc, Lexington, Ky.
g. Statview 5, SAS Institute Inc, Cary, NC.

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