Analgesic effects of carprofen and liposome-encapsulated butorphanol tartrate in Hispaniolan parrots (*Amazona ventralis*) with experimentally induced arthritis

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Objective—To evaluate the microcrystalline sodium urate (MSU) method for inducing arthritis in parrots and to compare the analgesic efficacy of long-acting liposome-encapsulated butorphanol (LEBT), carprofen, or a combination of both.

Animals—20 Hispaniolan parrots.

Procedures—MSU was injected into a tibiotarsal-tarsometatarsal (intertarsal) joint to induce arthritis (time 0). Four treatments were compared (LEBT [15 mg/kg, SC] administered once at time 0; injections of carprofen [3 mg/kg, IM, q 12 h] starting at time 0; administration of LEBT plus carprofen; and a control treatment of saline [0.9% NaCl] solution). Weight load testing and behavioral scoring were conducted at 0, 2, 6, 26, and 30 hours.

Results—Injection of MSU into the intertarsal joint induced arthritis, which resolved within 30 hours. Treatment with LEBT or LEBT plus carprofen resulted in significantly greater weight-bearing load on the limb with induced arthritis, compared with the control treatment. Treatment with carprofen alone caused a slight but nonsignificant improvement in weight-bearing load on the arthritic limb, compared with the control treatment. Behaviors associated with motor activity and weight bearing differed between the control and analgesic treatments.

Conclusions and Clinical Relevance—Butorphanol was an effective treatment for pain associated with arthritis, but carprofen administered every 12 hours was insufficient. Injection of MSU to induce arthritis in a single joint was a good method for evaluating tonic pain in parrots, and measurement of the weight-bearing load was accurate for assessment of arthritic pain; however, behavioral changes associated with pain were subtle. (*Am J Vet Res* 2009;70:1201–1210)

Administration of analgesics for conditions considered painful in humans is regarded as a standard of veterinary practice for all vertebrate species.[1] The clinical management of pain is an essential part of avian medicine and surgery. Evaluating analgesic efficacy in animals is challenging because objective measures of behavior associated with pain are difficult to define for nonverbal species. This is particularly true in less traditional companion animal species, such as birds, reptiles, amphibians, and fish.[1] Many avian species are commonly kept as companion and laboratory animals, and there is an urgent need to understand and manage pain in birds. In mammals, multimodal analgesia through systemic administration of a combination of opioids and NSAIDs is more effective than administration of either drug class individually.[2] The multimodal approach is clinically applied to birds, but to our knowledge, it has not been evaluated in controlled scientific studies. Opioids are the most effective class of analgesic drugs for the treatment of perioperative pain. Butorphanol tartrate, a mixed opioid agonist-antagonist with κ-opioid receptor agonism and μ-opioid receptor antagonism, is currently considered the analgesic drug of choice for management of acute and chronic pain in birds.[3] Pharmacodynamics and pharmacokinetics of

Abbreviations

COX	Cyclooxygenase
LEBT	Liposome-encapsulated butorphanol tartrate
MSU	Microcrystalline sodium urate

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butorphanol determined by use of African grey parrots (*Psittacus erithacus*) and Hispaniolan parrots (*Amazona ventralis*) suggest that the commercially available tartrate form administered IM may need to be repeated every 2 to 4 hours to maintain serum concentrations and analgesic effects.[4–6] A liposomal formulation is not yet commercially available but would be appropriately suited for use because a single administration of 15 mg/kg in parrots provides analgesic effects for a minimum of 72 hours.[5]

A broad tissue distribution of COX has been reported in chickens.[7] The mechanism of action of NSAIDs is attributed to the inhibition of COX activity. Carprofen is an NSAID licensed for use in dogs, and it is commonly used for the treatment of inflammatory conditions such as arthritis and as a perioperative analgesic. In a clinical evaluation of carprofen, dogs with osteoarthritis had significant improvement in the ground reaction forces of the arthritic limb.[8] In another study[9] in dogs with MSU-induced synovitis, carprofen administration resulted in an improvement in peak vertical ground reaction forces. Carprofen (1 mg/kg, SC) can improve the walking rate of lame chickens.[10] In another study,[11] lame broiler chickens preferentially selected feed mixed with carprofen over unmedicated feed and had improvement in their gait, with therapeutic carprofen concentrations of 8.3 µg/mL of plasma, although high concentrations were needed in the feed to achieve this plasma concentration.[11] A minimum effective carprofen dosage of 30 mg/kg, IM, is effective in chickens with arthritis, as determined by use of the MSU method for inducing articular pain.[12] However, this dosage is 6 and 10 times higher than the therapeutic dosages for rats and dogs, respectively, and administration of NSAIDs (including carprofen) at high dosages can cause renal histopathologic changes in birds.[13–15] The study reported here used the clinically applied dosage for parenteral administration of carprofen to pet birds (3 mg/kg).[16]

The MSU method of inducing articular pain is a reliable technique for inducing acute inflammatory pain that mimics the pain of naturally developing articular gout in mammals, chickens, and pigeons.[17–30] Intra-articular injection of MSU in chickens causes joint inflammation with sensitization of C fiber nociceptors in the joint capsule.[31] There is also a neurogenic depletion of substance P from peripheral nerve fibers in the synovium.[28] In the study reported here, our intent was to inject MSU into the tibiotarsal-tarsometatarsal (intertarsal) joint to induce temporary, self-resolving arthritis.

Pain-related behaviors have been identified in chickens (standing on 1 limb, limping, and excessive resting) that can be quantitatively measured by use of scan sampling.[12,24,29] Similar behaviors were included during development of an arthritis pain ethogram for Hispaniolan parrots, and we included additional behaviors of perching birds that may be affected by arthritis and pain.

Birds respond to stressors, such as pain, through various mechanisms. During periods of stress, production of cortisol and corticosterone is increased, and these hormones can be measured in the feces of birds.[32,33] Concentrations of hormone metabolites in feces reflect the cumulative secretion and elimination of hormones over several hours, and repeated collection of samples from the same bird is possible without affecting its behavior or endocrine status. Analysis of fecal corticosterone concentrations during the period of experimentally induced arthritis was an additional method for use in quantifying and comparing the stress of arthritic pain among treatments.

Therefore, the objectives of the study reported here were to evaluate the MSU method for inducing arthritis in Hispaniolan parrots, as determined by use of a weight-bearing load test designed for use in perching avian species; to develop and quantify a species-specific ethogram for measuring behavioral changes associated with arthritic pain; to determine fecal corticosterone concentrations in parrots with arthritic pain that were receiving analgesic treatments; and to compare the analgesic efficacy of LEBT, carprofen, or a combination of both drugs.

Materials and Methods

Animals—A uniform population of 20 adult (age range, 2 to 18 years; mean ± SEM, 4.80 ± 0.94 years) Hispaniolan parrots with a mean body weight of 266.60 ± 4.96 g but of unknown sex were used in the study. All parrots were part of the teaching and research flock at the School of Veterinary Medicine, University of Wisconsin, Madison, and were healthy during the study. Parrots were housed at the Charmany Instructional Facility, where they were maintained in flocks of 4 or 5 parrots in large rooms (11.2 m^2). Parrots were maintained on a cycle of 12 hours of light and 12 hours of darkness, fed a commercial pelleted diet[a] formulated for psittacine birds, and provided water ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee of the School of Veterinary Medicine, University of Wisconsin, Madison.

Parrots were allocated into period groups (5 parrots/period) 2 weeks prior to the study; the 5 parrots in each period were housed separately in adjacent standard stainless-steel laboratory cages (61 × 61 × 63.5 cm). There was a perch and a hanging toy inside each cage.

Preparation of MSU—Sodium urate monohydrate crystals were prepared in accordance with a method described elsewhere.[34] Briefly, 40 mL of 1N NaOH was added to sterile water for irrigation, additional sterile water was added to achieve a final volume of 1,600 mL, and then pH of the solution was adjusted to 12. The alkaline solution was boiled with stirring; 8 g of uric acid was added to the boiling solution. The solution was then boiled for an additional 5 minutes and removed from the heat. Additional 1N NaOH was added until the solution cleared. The solution was allowed to sit at 23°C for 48 hours; the pH was adjusted to 8 and allowed to sit at 23°C for another 24 hours. After 72 hours, the crystals were grossly light, flocculent, and white and floated easily in the solution. Microscopically, the crystals appeared as rods, needles, or aggregates of small needles. The solution was allowed to sit for another 24 hours. The entire contents of a flask were then poured

into 50-mL centrifuge tubes, which were centrifuged at 693 × *g* for 15 minutes. The supernatant was decanted, and the crystals were washed in 250 mL of sterile saline (0.9% NaCl) solution. Crystals were centrifuged again in 50-mL centrifuge tubes (693 × *g* for 20 minutes), the supernatant was decanted, and the crystals were resuspended in 200 mL of sterile saline solution in a sterile flask. The flask was capped with aluminum foil and autoclaved at 93.3°C for 2 hours. Crystals were pipetted by use of a 25-mL pipet and continuous stirring in 7-mL aliquots into sterile 2-dram vials. Each vial was centrifuged at 390 × *g* for 15 minutes; the supernatant was decanted and measured, and 50% of the fluid was replaced for resuspension to yield an 8% sodium urate crystal solution suspended in sterile saline solution.

Preparation of LEBT—A dehydration-rehydration vesicles method was used, as described in another study.[5] Briefly, 59.7 mg of powdered butorphanol tartrate[b] was dissolved in 10 mL of 1mM sodium citrate buffer (pH, 4.0). The solution was sterilized by use of a 0.22-µm filter.[c] A film of 80µM egg phosphatidylcholine was dried onto the walls of an 18-mm test tube by use of a rotating water bath (set at 37°C) connected to a suction device. The dried film of egg phosphatidylcholine was overlaid with 5 mL of the butorphanol tartrate solution. The mixture was sonicated for 10 minutes. The resulting mixture was transferred to sterile round-bottomed flasks. The mixture was frozen by use of a bath containing isopropanol and dry ice. The round-bottomed flasks were attached to a freeze dryer and freeze-dried overnight. The flasks were removed from the freeze dryer after 24 hours, sealed, and stored at –20°C until rehydrated. Liposomes were rehydrated by the addition of 0.5 mL of sterile water for irrigation; liposomes were allowed to rehydrate for 0.5 hours. Saline-acetate buffer (9.5 mL of sterile saline solution with 10mM acetate buffer [pH, 4.0]) was added to the rehydrated liposomes. The liposomes were transferred to a 15-mL conical centrifuge tube and centrifuged at 10,000 × *g* for 10 minutes. Excess buffer was removed, and the resulting pellet of liposomes (yield of approx 0.5 mL of packed liposomes) was washed twice and resuspended in 2 mL of fresh saline-acetate buffer. Liposomes were stored at 5°C until quantitated and used in experiments. Liposome preparations containing butorphanol tartrate were quantitated by suspending 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 resulting solution was placed in a cuvette, and absorbance was determined at 281 nm. Saline-acetate buffer suspended in the same solvent solution was used as a blank sample.

Experimental induction of arthritis—All 20 parrots received an intra-articular injection of MSU into the intertarsal joint of one of the pelvic limbs. Anesthesia was induced in the parrots via mask administration of 5% isoflurane gas in 1 L of oxygen/min. Parrots were then intubated with a 2.5-mm endotracheal tube, and anesthesia was maintained by administration of 2% to 3% isoflurane gas in 1 L of oxygen/min by use of a Bain circuit nonrebreathing system. Each parrot was weighed, and ophthalmic ointment was topically applied to provide corneal protection. Parrots were positioned in dorsal recumbency on a heated pad. A steel identification band was located on 1 pelvic limb of each parrot (the left limb, except in 2 parrots); thus, the opposite limb (ie, the right limb in 18 parrots and the left limb in 2 parrots) was used for injection. Feathers were gently plucked from the distal region of the tibiotarsus and proximal region of the tarsometatarsus of the selected limb, and the area was aseptically prepared.

After preparing the site, a 23-gauge needle was inserted into the tibiotarsal-tarsometatarsal (intertarsal) joint space toward the plantar aspect of the joint, and 0.1 mL of 8% sodium urate crystal suspension was injected. The joint was flexed and extended for 15 to 30 seconds to distribute the MSU within the joint space. Prior to cessation of anesthesia, each parrot was administered the first dose of the experimental treatment. Once all materials were administered, isoflurane was discontinued; each parrot was placed in a recovery cage when the righting reflex was observed.

Study design—Parrots were randomly assigned to 4 treatments. Time of injection of the MSU and initial experimental treatment was designated as time 0. The 4 treatments were a single SC injection of LEBT (15 mg/kg) at time 0; injections of carprofen[d] (3 mg/kg, IM, q 12 h) beginning at time 0; concurrent administration of LEBT (15 mg/kg, SC, once) and carprofen (3 mg/kg, IM, q 12 h) beginning at time 0; and a control treatment of saline solution (0.2 mL, IM, q 12 h), which was similar to the volume, route, and dosage interval for carprofen. The LEBT was administered SC between the body wall and proximal medial femoral region by use of a 22-gauge needle. Carprofen or saline solution was administered IM in a pectoral muscle, with the site alternating between the left and right sides for each subsequent injection.

A complete crossover design was used; there were 4 periods with a minimum washout period of 2 weeks between subsequent treatments. During the experimental periods, each parrot within a group was assigned 1 of the 4 treatments. Each parrot received all 4 treatments, but the order of treatments for each parrot was randomly determined. Regardless of treatment, all parrots were manually restrained every 12 hours to simulate handling of parrots receiving the carprofen treatment.

To our knowledge, the MSU method had not been used in psittacine birds; therefore, it required thorough validation. This method has been used in a number of other species, including human volunteers who can report the nature and limited duration of pain; therefore, although the AVMA does not recommend inclusion of a placebo control group in a study such as this,[35] we believed it was appropriate to use untreated control parrots in the study. In addition, the protocol included a requirement to remove any parrot from the study when the bird had signs of excessive pain (such as loss of appetite or reluctance to move about the cage for periods of > 1 hour) following MSU injections to induce arthritis; parrots removed because of excessive pain were to be provided additional analgesia and supportive care.

Assessment of pain and analgesia—The response to treatment was evaluated by use of 3 methods.

Weight load testing

An incapacitance meter[e] was custom designed for use with Hispaniolan parrots. The standard rodent foot-

pads were converted into 2 perching rods (1 for each foot). A black plastic box (27 × 11.5 × 23 cm) with a transparent front and a hinged door was placed over the perching rods to limit each parrot's movements during measurements. The incapacitance meter used dual-channel weight averaging, which enabled testing of both limbs simultaneously. If a parrot shifted from one foot to the other foot, the unit recorded the average weight for each foot during a predetermined test period of 30 seconds. Weight load values were recorded for 3 consecutive 30-second intervals. Prior to onset of the study, a 2-week acclimation period was used to condition the parrots to perch inside the test box for 2- to 5-minute intervals. Baseline weight load tests were performed for each parrot 1 week before intra-articular injection of MSU. After arthritis was induced, weight load data were collected at 2, 6, 26, and 30 hours. During the first 3 treatment periods, each parrot received carprofen and saline solution treatments every 12 hours for a total of 96 hours and were evaluated at 50, 54, 74, and 98 hours. Data analyzed while the study was in progress indicated that the duration of the induced arthritis was < 30 hours; thus, for the fourth treatment period, treatments and evaluations were discontinued at 30 hours. Data collected at > 30 hours were not included in the final analysis.

Data for each parrot at each time point were calculated to reflect the difference in weight bearing between the MSU-injected and the noninjected limb by use of the following equation: change in weight bearing = (mean weight bearing of noninjected limb – mean weight bearing of noninjected limb at baseline) – (mean weight bearing of injected limb – mean weight bearing of injected limb at baseline). This equation was used to account for variation in weight bearing among parrots prior to induction of arthritis. By use of this method, higher scores indicated a decrease in weight bearing on the injected limb and an increase in weight bearing on the noninjected limb, which was used as an indicator of pain.

Assessment of voluntary and motivated behavior

Prior to data collection, an ethogram was developed to evaluate and quantify typical behavior of a parrot when housed alone in a laboratory cage setting. In addition, the ethogram was expanded to include a set of behaviors associated with articular pain within the same environmental context. Thirty-five behaviors were defined and scored or measured in terms of duration, intensity, or frequency. The Spearman rank order coefficient was applied to all behavior data collected during the first 30 hours of each treatment period to identify behaviors significantly affected by the experimentally induced arthritis (**Appendix**). A food reward of a fresh grape was placed on a hook at the upper left corner of the cage at the start of each behavioral evaluation session. Parrots were conditioned to this food reward, and it was used to assess motivated climbing behavior during the study. To avoid effects of observers on behavior, parrots were remotely video recorded[f] and an observer was not present, except during the introduction of the grape. Parrots were recorded in their cages for 15-minute periods prior to intra-articular injection of MSU and at 2, 4, 6, and 30 hours; recordings were obtained immediately after weight load testing. During the first 3 treatment periods, monitoring continued every 12 hours for a total of 98 hours. Because the induced arthritis was determined to resolve by 30 hours, behavioral observation was discontinued at 30 hours during the final treatment period. Therefore, only data collected up to 30 hours were used in the analysis.

Fecal corticosterone concentration

A fecal sample was collected each time that a weight-bearing perching assessment was performed. Samples were frozen at –70°C until analysis. Fecal samples collected at 0, 6, and 26 hours were analyzed by use of a radioimmunoassay kit at an endocrine laboratory[g] to determine corticosterone concentrations.

Statistical analysis—The weight-bearing and fecal corticosterone data were analyzed by use of commercially available software.[h] A repeated-measures ANOVA was used, with fixed effects of treatment, treatment period, time, and all associated interactions. Parrot was considered a random effect to account for correlations among observations on the same parrot. Residuals resulting from the fitted model were verified to be acceptably normally distributed and did not have evidence of heteroskedacity. Pairwise comparisons of the treatments within each time point and for all time points were performed by use of the Tukey *P* value correction to account for multiple comparisons. Values were considered significant at $P \leq 0.05$.

Behavioral data were not normally distributed; therefore, nonparametric statistical tests were used in the analysis. These data were analyzed by use of commercially available software.[i] To reduce the number of behaviors for analysis, the Spearman rank order correlation was used to identify and remove ordinal behaviors that were substantially similar. For all remaining behaviors, treatments were compared on a pairwise basis separately for each time point by use of a 2-tailed Wilcoxon signed rank test. Again, values were considered significant at $P \leq 0.05$.

Results

Animals—Injection of MSU into 1 intertarsal joint successfully induced a temporary monoarticular arthritis in parrots, which resolved within 30 hours. One parrot completed the first treatment period but was subsequently removed from the study because its perching behavior became unpredictable.

Weight load testing—Parrots that received the control treatment after experimental induction of arthritis had significantly ($P = 0.01$) less weight loading on the affected limb at 6 and 26 hours, compared with the weight loading at time 0 (**Figure 1**). At 30 hours, the parrots still favored the noninjected limb, but because of a large amount of individual variation, the values for the injected and noninjected limbs did not differ significantly ($P = 0.06$). The value at 30 hours for parrots receiving the control treatment did not differ significantly from the baseline value.

A significant ($P = 0.03$) overall treatment effect was detected. Compared with results for the control treatment, LEBT ($P = 0.04$) or the combination of LEBT plus carprofen ($P = 0.05$) resulted in significantly more weight-bearing load on the limb with experimentally

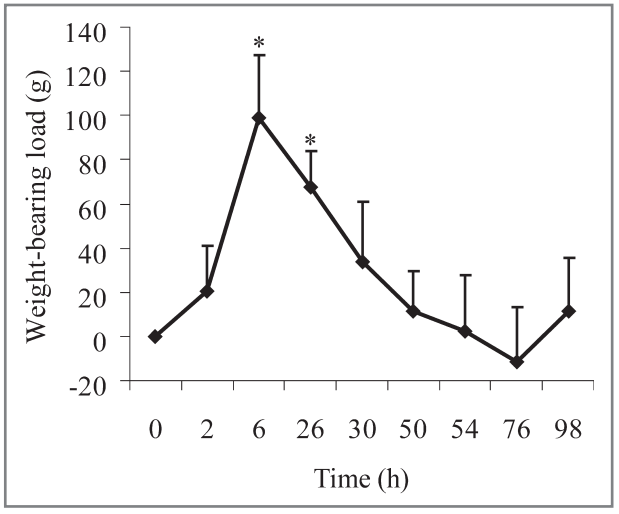

Figure 1—Mean ± SD change in weight-bearing load determined for a 98-hour period after injection of MSU into 1 tibiotarsal-tarsometatarsal (intertarsal) joint (time 0) in each of 19 Hispaniolan parrots (*Amazona ventralis*); the parrots did not receive any analgesics (control treatment). Change in weight-bearing load for each time point was calculated as follows: (mean weight bearing of noninjected limb – mean weight bearing of noninjected limb at 0 hours) – (mean weight bearing of injected limb – mean weight bearing of injected limb at 0 hours). Higher values indicate an increase in weight bearing on the noninjected limb and a decrease in weight bearing on the limb with experimentally induced arthritis. *Value differs significantly ($P \leq 0.05$) from the value at 0 hours.

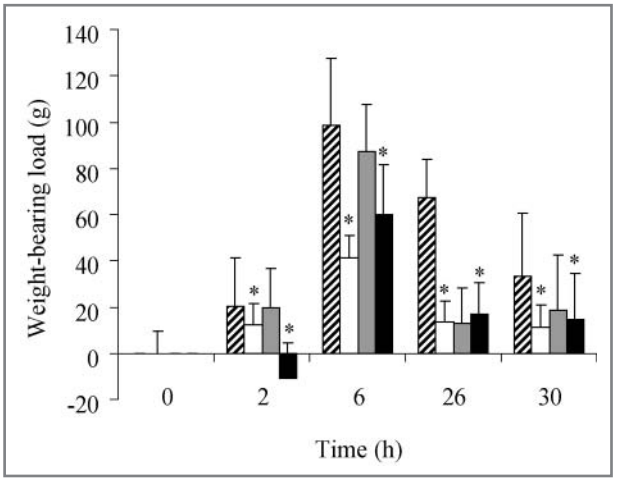

Figure 2—Mean ± SD change in weight-bearing load in parrots during a 30-hour period after injection of MSU into 1 intertarsal joint (time 0) followed by administration of 4 treatments. Treatments were as follows: control, 0.2 mL of saline (0.9% NaCl) solution, IM, every 12 hours beginning at time 0 (diagonal-striped bars); LEBT, 15 mg/kg, SC, once at time 0 (white bars); carprofen, 3 mg/kg, IM, every 12 hours beginning at time 0 (gray bars); and concurrent administration of LEBT (15 mg/kg, SC, once) plus carprofen (3 mg/kg, IM, q 12 h) beginning at time 0 (black bars). Data represent results for 19 parrots, except for the LEBT plus carprofen treatment, in which data represent results for 20 parrots. *Within a time point, value differs significantly ($P \leq 0.05$) from the value for the control treatment. *See* Figure 1 for remainder of key.

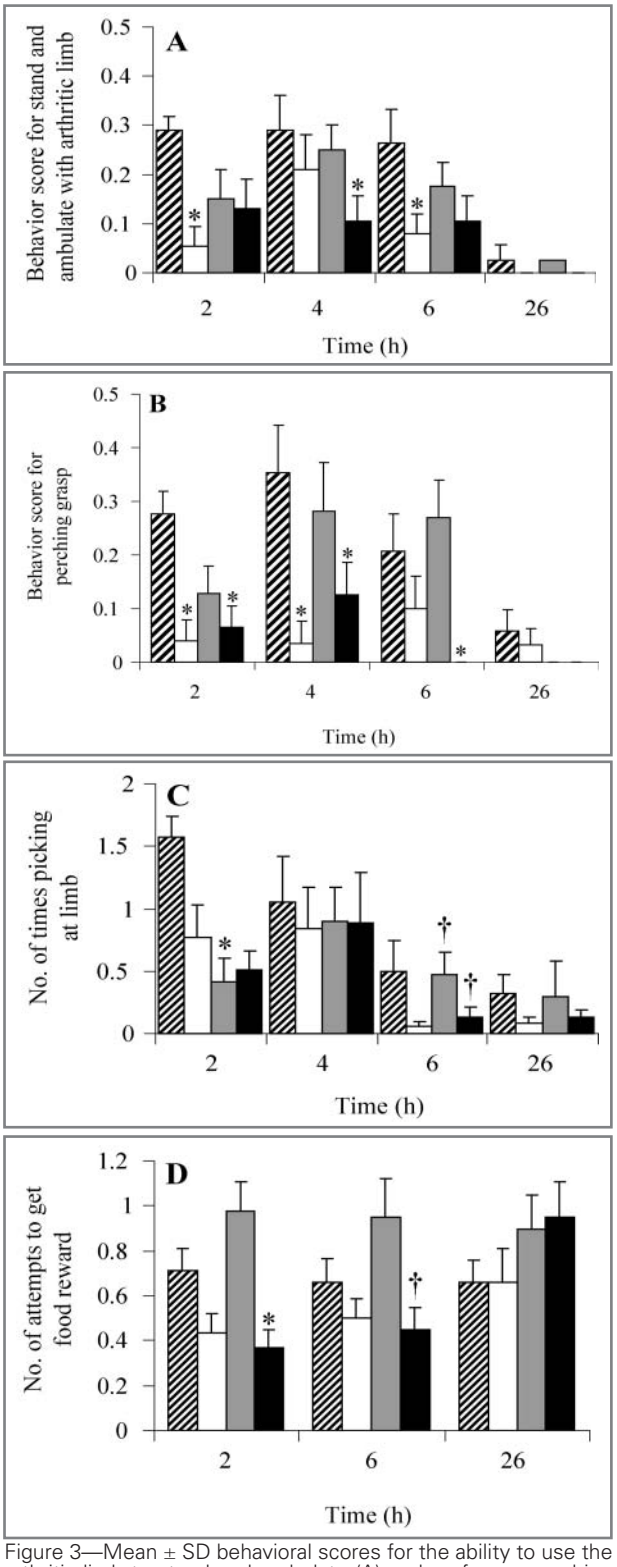

Figure 3—Mean ± SD behavioral scores for the ability to use the arthritic limb to stand and ambulate (A) and perform a perching grasp (B), the number of times picking at the arthritic limb (C), and number of attempts to get a food reward (ie, a grape; D) during a 15-minute observation period in parrots after injection of MSU into 1 intertarsal joint (time 0) and subsequent administration of 4 treatments. Scores were assigned as a dichotomous result of standing and ambulating with the arthritic limb (0 = No; 1 = Yes) and perching grasp (0 = Both feet; 1 = Only 1 foot). †Within a time point, value differs significantly ($P \leq 0.05$) from the value for the carprofen treatment. *See* Figure 2 for remainder of key.

induced arthritis (**Figure 2**). Treatment with LEBT or LEBT plus carprofen was equally effective ($P = 0.99$) in improving weight-bearing load of the arthritic limb. Carprofen treatment improved weight-bearing load on the arthritic limb, compared with results for the control treatment, but the values did not differ significantly ($P = 0.30$) when all time points were included. There was also a significant ($P = 0.01$) effect of time, such that all treatments had the lowest weight-bearing load for the arthritic limb at 6 hours. Analysis of the data did not reveal significant interactions or effects of treatment period.

Behavior assessment—Clear differences existed between the control and analgesic treatments regarding behaviors associated with motor activity and weight bearing on the arthritic limb (**Figure 3**). Differences in behaviors were summarized (**Table 1**). Parrots were significantly more likely to stand and ambulate normally at 2 hours ($P = 0.01$) and 6 hours ($P = 0.03$) using the arthritic limb when they received LEBT, compared with results when they received the control treatment; however, values did not differ significantly between LEBT and control treatments at 4 hours. Treatment with LEBT plus carprofen caused a nonsignificant improvement in ambulation at 2 hours, compared with results for the control treatment. At 4 hours, treatment with LEBT plus carprofen caused a significant ($P = 0.04$) improvement in the ability to stand and ambulate normally, compared with results for the control treatment. Treatment with carprofen alone resulted in behavior similar to that for the control treatment at 2, 4, and 6 hours. There were no significant differences in ambulation behaviors among treatments at 26 hours (a time when the arthritis was resolving).

Diminished ability to perch on both feet was detected more frequently for the control treatment. The LEBT and LEBT plus carprofen treatments were significantly more likely to result in parrots perching on both feet, compared with results for the control treatment, at 2 ($P = 0.02$ for both treatments) and 4 ($P = 0.01$ and $P = 0.05$ for LEBT and LEBT plus carprofen, respectively; Figure 3) hours. By 6 hours, only the LEBT plus carprofen treatment was significantly ($P = 0.01$) more likely to result in perching on both feet, compared with results for the control treatment. There were no significant differences among treatments at 26 hours.

Feather ruffling was detected significantly less frequently when parrots received LEBT plus carprofen, compared with results when parrots received the control treatment, at 2 ($P = 0.04$) and 6 ($P = 0.05$) hours. In contrast, the LEBT and control treatments consistently resulted in feather ruffling more frequently at 2 hours, compared with results for the caprofen or LEBT plus carprofen treatments. There were no significant differences in feather ruffling among treatments at any other time point.

When parrots received the control treatment, they used their beak to pick at the arthritic limb more frequently than did parrots when administered the other treatments; however, only carprofen treatment resulted in significantly ($P = 0.03$) less picking at the arthritic limb at the 2-hour time point (Figure 3). Limb-picking behavior was similar for all treatments at 4 hours, but at 6 hours, carprofen resulted in significantly ($P = 0.03$) less picking at the arthritic limb, compared with results for LEBT plus carprofen.

Table 1—Differences in behavioral scores for the control treatment (0.2 mL of saline [0.9% NaCl] solution, IM, q 12 h beginning at time 0), compared with scores for the LEBT (15 mg/kg, SC, once at time 0), carprofen (3 mg/kg, IM, q 12 h beginning at time 0), and LEBT plus carprofen (concurrent administration of LEBT [15 mg/kg, SC, once] and carprofen [3 mg/kg, IM, q 12 h] beginning at time 0) treatments in Hispaniolan parrots (*Amazona ventralis*) after injection of MSU into 1 intertarsal joint (time 0) and subsequent administration of the 4 treatments.

Time (h)	Control vs LEBT	Control vs carprofen	Control vs LEBT plus carprofen
2	Perching grasp* ($P = 0.020$) Perching posture* ($P = 0.044$) Time spent eating food* ($P = 0.001$) Visits to food dish† ($P < 0.001$) Stand and ambulate with arthritic limb* ($P = 0.007$)	Time spent eating food reward* ($P = 0.044$) Picking at arthritic limb with beak* ($P = 0.032$)	Perching grasp* ($P = 0.020$) Locomotion† ($P = 0.037$) Feathers ruffled* ($P = 0.043$) Ruffle feathers* ($P = 0.05$) Attempts made to get food reward* ($P = 0.049$)
4	Perching grasp* ($P = 0.001$) Perching posture* ($P = 0.001$)	Rub beak on wood† ($P = 0.049$)	Stand and ambulate with arthritic limb* ($P = 0.035$) Perching grasp* ($P = 0.045$)
6	Use noninjected limb to hold food reward while eating it† ($P = 0.049$) Stand and ambulate with arthritic limb* ($P = 0.03$)	—	Perching grasp* ($P = 0.005$) Feathers ruffled* ($P = 0.05$) Use noninjected limb to hold food reward while eating it† ($P = 0.027$)
26	Time spent eating* ($P = 0.040$) Rub beak on metal† ($P = 0.046$)	—	—
30	Hang from top of cage† ($P = 0.006$) Rub beak on wood† ($P = 0.025$)	Time from introduction of food reward to first contact with reward* ($P = 0.006$) Rub beak on metal† ($P = 0.038$)	Hang from top of cage† ($P = 0.020$)

Behavioral scores were determined during a 15-minute period at 0, 2, 4, 6, 26, and 30 hours. The food reward was a grape. Values were considered significant at $P \leq 0.05$.

*Within a comparison, the control treatment had the higher mean score. †Within a comparison, the analgesic treatment had the higher mean score.

— = Within a comparison, no significant differences in behavioral scores between treatments.

When provided with a grape, more attempts were required to reach the grape by parrots when they received the control or carprofen treatments, compared with results when parrots received LEBT or LEBT plus carprofen (Figure 3). When parrots received LEBT plus carprofen, they required significantly fewer climbing attempts to reach the grape at 2 hours, compared with the number of attempts needed when administered the control treatment ($P = 0.05$). They also required fewer climbing attempts at 6 hours, compared with the number of attempts needed when administered carprofen ($P = 0.04$). There were no other significant differences among treatments at any other time point.

Only carprofen resulted in parrots attempting to eat the grape with the arthritic limb at 2 hours, which differed significantly ($P = 0.04$) from results for the control, LEBT, or LEBT plus carprofen treatments in which parrots stood on the arthritic limb while using the noninjected foot to manipulate the grape. Use of the arthritic limb to manipulate the grape was not detected for the control treatment until 26 hours, whereas the LEBT and LEBT plus carprofen treatments resulted in parrots using the affected limb to manipulate the grape by 6 hours.

Fecal corticosterone concentrations—Baseline fecal concentrations of corticosterone were highly variable among parrots, with a range of 63 to 3,677 ng/g of dry feces (median, 580 ng/g of dry feces). The mean ± SD concentration of corticosterone in fecal samples collected at 6 and 26 hours was significantly ($P = 0.01$) greater than the mean baseline concentration, but there was no significant difference among the treatments. Regardless of treatment, fecal cortisosterone concentrations were significantly ($P = 0.01$) higher at 6 hours than at 26 hours.

Discussion

Injection of MSU into a single intertarsal joint of Hispaniolan parrots induced an experimental monoarticular arthritis that was self-limiting and resolved within 30 hours. Weight bearing on the limb with experimentally induced arthritis without administration of an opioid or NSAID was reduced at 2, 6, and 26 hours but returned to baseline values at 30 hours. This method for inducing tonic pain mimics the clinical condition of articular gout in avians in which chronic uricemia leads to accumulation of urate crystals in the joints, which results in a non–weight-bearing condition.[36] The physiologic response in avians is similar to that in mammals in that the crystals cause pain associated with inflammation; this causes recruitment of polymodal nociceptors with afferent signals transmitted by C-type fibers, which are small-diameter, unmyelinated fibers with rapid conduction velocities, in addition to sleeping nociceptors, which are activated following a short period of inflammation.[21–23]

In many of the studies on experimentally induced arthritis in chickens, investigators have evaluated the birds during the first 24 hours after induction, and few used quantitative measurements. Results of ground reaction forces for objective gait analysis in chickens have high variability, which limits the usefulness of the technique in that species.[37] In mammals, weight load in arthritic limbs is consistent, reproducible, and considered a reliable objective index for assessing the severity of arthritic pain in several species.[8,9,38] In the study reported here, weight-bearing load in the pelvic limb was objectively measured by use of an incapacitance meter adapted for use in parrots to evaluate the ecologically relevant behavior of perching. The technique tested both limbs simultaneously and provided a reliable and repeatable measurement of weight load in unrestrained parrots. Data on weight-bearing load were more sensitive to the effects of experimentally induced arthritis than were the other methods evaluated in this study, and it was useful in detecting changes in weight bearing at 26 hours. In contrast, behavioral observations of lameness were only detected when lameness was severe during the first 6 hours after injection of MSU.

When treated with LEBT or LEBT plus carprofen, parrots had improved weight-bearing capacity in the limb with experimentally induced arthritis, compared with parrots when they received the control treatment that did not contain any analgesic. Parrots were able to bear weight on both limbs, similar to baseline measurements obtained before induction of arthritis. Long-acting liposomal butorphanol can provide effective and constant butorphanol concentrations throughout the course of a study.[5] The commercially available form of butorphanol would be expected to provide similar analgesia but would need to be administered every 2 to 4 hours, as determined on the basis of analgesimetric studies[4,39] and pharmacokinetic studies.[6,40]

Carprofen increased the weight-bearing load of the arthritic limb, but not significantly, for the 30-hour period. The weight-bearing load when carprofen was administered was greater at 2 and 26 hours than at 6 and 30 hours, which were longer intervals after carprofen injection (carprofen was injected at 0, 12, and 24 hours; Figure 2). Carprofen was superior to LEBT for reducing feather picking on the arthritic limb (Figure 3). This may have been attributable to the effect of carprofen on pruritus associated with inflammation that was not affected by LEBT.[41] The authors believe that carprofen may still be an effective analgesic for parrots, but the dosage and frequency of administration may need to be increased. The dosage of carprofen (3 mg/kg) was derived from anecdotal references for clinical application for pet birds, but to our knowledge, there have been no pharmacokinetic studies in any pet bird species to evaluate carprofen. In another study,[11] therapeutic plasma concentrations of 8.3 µg of carprofen/mL were obtained when lame chickens consumed drug-containing feed for 24 hours. However, it is difficult to extrapolate a recommendation for a treatment dosage on the basis of that study.[11]

The lack of additional improvement in weight bearing when the same dosage of carprofen was administered in addition to LEBT, versus LEBT alone, was additional support for the ineffectiveness of the carprofen dosage for providing analgesia in parrots. In mammalian studies,[2,42] multimodal analgesia through systemic administration of a combination of opioids and NSAIDs is more effective than administration of either drug class alone. In the study reported here, we were not able to

evaluate multimodal analgesia because carprofen was not effective at the dosage evaluated. Additional studies are needed to determine an effective dosage for carprofen in parrots while evaluating the potential adverse effects of high-dose NSAIDs on renal pathologic changes or potential gastrointestinal tract bleeding.

An ethogram is a systematic inventory of behaviors of a species, with the behaviors organized into categories. In this study, particular attention was given to any behavior that may have been affected by pain or lameness. Analysis of 35 behaviors scored during > 30 hours revealed few behaviors that differed significantly among treatments. Observable behaviors directly related to weight bearing and perching could be used to differentiate among treatments within the first 6 hours. When administered LEBT, parrots scored better (less lame than when they received the control treatment) than when administered carprofen alone, which directly correlated with the quantitative weight-bearing data, although the weight-bearing measurements were more sensitive and could differentiate among treatments for up to 26 hours. The motivational behavioral score that used attempts to get a food reward was the most useful at 2 hours after injection of MSU because treatment with LEBT plus carprofen was more likely to result in parrots obtaining and eating the grape. Concerns that butorphanol may have a sedative effect during the early treatment phase when plasma concentrations were highest were diminished with this finding, although food motivation was not specifically used to score sedation. It was also evident that when parrots were administered LEBT, they were more likely to stand on the arthritic limb while using the unaffected foot to manipulate the food reward, whereas when parrots were administered carprofen alone, they preferred to use the arthritic limb to hold the food reward. We believe that painful arthritis inhibited these parrots from placing their full weight on the affected limb.

Behaviors not directly related to arthritic pain were much more difficult to isolate, and few were correlated with the detection or severity of arthritis. Feather ruffling is believed to be a behavior associated with discomfort in parrots, and it was detected for the control treatment, whereas LEBT plus carprofen resulted in a decrease in feather ruffling at 2 and 6 hours. In contrast, when parrots received LEBT alone, they had an increase in feather ruffling at 2 hours. In another study,[5] use of LEBT resulted in high plasma concentrations of butorphanol at 2 hours; therefore, the association between feather ruffling and opioid effects in this species would need further investigation. Additional studies would enable researchers to gain better understanding of the behavioral responses of parrots to pain and to common analgesics.

Fecal corticosterone concentrations did not differ significantly among treatments at the time points analyzed; 0, 6, and 26 hours were selected to reflect the period prior to treatment, the period of greatest arthritic pain, and a time near recovery, respectively. The lack of significant differences among treatments was attributable to the large range of values among the parrots. There were many confounding factors in the study design that could have influenced stress responses of the parrots. Despite the prestudy conditioning period used to acclimate the birds to the testing environment and procedures, the introduction of variables (such as restraint, anesthesia, and anesthetic recovery) would be stressful events. Additionally, all birds had some degree of painful arthritis at some point during the study, which would be reflected in a cumulative assay. Fecal concentrations of corticosterone were highest at 6 hours when experimentally induced arthritis was pronounced, but without other studies in this species to correlate fecal corticosterone concentrations with ACTH stimulation, it is impossible to predict when concentrations would be expected to peak in feces after a stressful incident.

Butorphanol can be an effective treatment for chronic pain associated with arthritis and is the treatment of choice for parrots with painful conditions, both acute and chronic. Additionally, MSU-induced arthritis is a good method for assessing tonic pain and analgesia in Hispaniolan parrots. Simultaneous measurement of weight-bearing loads by use of the perches for each foot provided an accurate, repeatable, and reliable method to quantify lameness associated with arthritic pain. Pain scales must be based on species-specific behaviors directly affected by the type of pain being monitored. Behavioral changes associated with pain, other than lameness, were subtle and difficult to measure in these captive parrots. Additional studies with Hispaniolan parrots and other psittacine species are needed to advance recognition of pain-associated behaviors.

a. Exact, Kaytee Products Inc, Chilton, Wis.
b. Butorphanol tartrate, Sigma Chemical Co, St Louis, Mo.
c. Gelman filters, Pall Corp, Ann Arbor, Mich.
d. Rimadyl injectable, 50 mg/mL, Pfizer Animal Health, Exton, Pa.
e. IITC model 600 incapacitance meter, IITC Life Science, Woodland, Calif.
f. Canon Optura 40, Canon USA Inc, Lake Success, NY.
g. Saint Louis Zoo Endocrinology Lab, Saint Louis Zoo, St Louis, Mo.
h. SAS, version 9.1.3, SAS Institute Inc, Cary, NC.
i. SPSS, version 11.1, SPSS Inc, Chicago, Ill.

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Appendix appears on the next page

Appendix

Scoring system for behaviors of Hispaniolan parrots (*Amazona ventralis*) with experimentally induced arthritis and receiving various analgesic treatments.

Behavior	Score
Voluntary	
Activity	0 = Moving around cage, 1 = Moving on perch, 2 = No activity
Inactive time	0 to 15 = No. of minutes parrot was inactive
Locomotion	0 = Both pelvic limbs, 1 = Only 1 pelvic limb, 2 = No movement
Perching posture	0 = Entire pelvic limbs visible, 1 = One full pelvic limb visible, 2 = Sitting on tarsometarsus of both pelvic limbs, 3 = Sitting on tarsometatarsus of 1 pelvic limb
Perching grasp	0 = Both feet, 1 = Only 1 foot
Stand and ambulate with arthritic limb	0 = No, 1 = Yes
Hang from top of cage	0 to infinity = No. of times parrot hung for > 10 seconds
Appearance	0 = Smooth feather, 1 = Slightly fluffed, 2 = Very fluffed; feathers sticking out
Feathers ruffled	0 = No, 1 = Yes
Feather ruffling	0 to infinity = No. of times parrot ruffled feathers
Preening	1 = With beak and feet, 2 = With beak only, 3 = None
Grooming	0 to infinity = No. of times parrot groomed
Rub beak on metal perches	0 = No, 1 = Yes
Rub beak on wood	0 = No, 1 = Yes
Attitude	0 = Alert, 1 = Signs of slight depression, 2 = Signs of depression
Use injected limb to hold food reward while eating it	0 = No, 1 = Yes
Use noninjected limb to hold food reward while eating it	0 = No, 1 = Yes
Visits to food dish	0 to infinity = No. of times to food and water
Time spent eating food	0 to 15 = No. of minutes
Picking at arthritic limb with beak	0 = No, 1 = Yes
Motivated*	
Attempts made to get food reward	0 to infinity = No. of attempts
Time from introduction of food reward to first contact with reward	0 to 15 = No. of minutes
First contact with food reward	0 to 15 = No. of minutes
Time spent eating food reward	0 to 15 = No. of minutes

*Motivated behaviors were those associated with obtaining a food reward; a grape was the food reward offered.