Journal of the American Association for Laboratory Animal Science
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Vol 52, No 5
September 2013
Pages 571–576

Effects of Multimodal Analgesia with Low-Dose Buprenorphine and Meloxicam on Fecal Glucocorticoid Metabolites after Surgery in New Zealand White Rabbits (*Oryctolagus cuniculus*)

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Despite the increasing use of rabbits as companion animals and models for biomedical research, rabbits have not been extensively studied to identify an efficacious postsurgical analgesic that does not cause systemic complications. The synergy of NSAID and systemic opioids is well-documented, and their combined use reduces the amount of either drug required for adequate analgesia. We measured fecal corticosterone metabolites (FCM) in rabbits after a minimally invasive vascular cut-down procedure. Rabbits received buprenorphine (0.03 mg/kg SC every 12 h for 3 d), meloxicam (0.2 mg/kg SC every 24 h for 3 d), buprenorphine–meloxicam (0.01 mg/kg–0.1 mg/kg SC every 24 h for 3 d), or a single dose of 0.5% bupivacaine (0.5 mL) infused locally at the incision site. By day 3 after surgery, buprenorphine, meloxicam, and bupivacaine groups showed elevated FCM levels, which continued to rise until day 7 and then gradually returned to baseline by day 28. In the buprenorphine–meloxicam group, FCM was relatively unchanged until day 3, when treatment was discontinued, and then began to rise. Rabbits in the buprenorphine–meloxicam group gained more weight over the 28-d study than did those in the other 3 treatment groups. This study shows that in rabbits low-dose buprenorphine administered with meloxicam effectively mitigates the FCM response that develops after surgery without the adverse effects associated with higher doses.

Abbreviations: COX, cyclooxygenase; FCM, fecal corticosterone metabolites.

Rabbits have gained popularity as companion animals and models for biomedical research because of their small size and easy temperament. They are widely used in research because of their physiologic similarity to humans and have been used extensively for studies in transgenics, immunology, cardiovascular and metabolic disorders, cancer, and development of new surgical techniques.[5,30] Companion rabbits frequently present for a number of surgical procedures including ovariohysterectomy or castration, gastrotomy or enterotomy for foreign-body removal, long-bone fracture repair, soft-tissue injuries, and dental or cutaneous abscesses.[12,26,30] Pain management in companion and laboratory animals is an ethical imperative for veterinarians, investigators, and animal caretakers. The Animal Welfare Act, Public Health Service policy, and the *Guide for the Care and Use of Laboratory Animals* mandate that procedures expected to cause more than slight or momentary pain require the appropriate use of pain-relieving measures, unless the withholding of analgesia is scientifically justified in an approved animal care and use protocol.[2,13,23] Furthermore, pain elicits an endocrine response cascade, including release of glucocorticoids, catecholamines and other stress-associated hormones that cause a variety of physiologic, metabolic, and inflammatory changes that may lead to organ dysfunction and confound research.[3,6,14] However, little primary literature documents an effective postsurgical analgesic regimen that is not associated with systemic complications or side effects, and veterinarians must rely on analgesic protocols extrapolated from other small mammals. This practice is not ideal because of the many physiologic, anatomic, and behavioral differences among species.

The use of opioids remains a major component of analgesic therapy, particularly in the treatment of moderate to severe postsurgical pain. Opioids exert their effect by inhibiting the transmission of nociceptive stimulation in the dorsal horn of the spinal cord, activating descending inhibitory pathways, inhibiting supraspinal afferents, and causing a decrease in the release of neurotransmitters in the spinal cord. Opioids commonly used in rabbits include butorphanol, buprenorphine, morphine, hydromorphone, oxymorphone, and fentanyl.[14] The most commonly used mixed agonist–antagonists are butorphanol and buprenorphine. The analgesic effect of butorphanol lasts about 3 h and is suitable for mild to moderate pain in rabbits, but the necessary frequency of administration makes it impractical for many situations. The analgesic effects of buprenorphine seem to last quite a bit longer than does butorphanol, persisting for 6 to 10 h after subcutaneous injection.[14] Although opioids are effective at controlling postoperative pain, side effects associated with their use can be significant. Buprenorphine in rabbits causes a marked decrease in arterial blood pressure, increased arterial carbon dioxide tension, and significant drop in respiratory rate and arterial oxygen tension resulting in mild hypoxemia.[14,27] This effect can be clinically important in animals at risk for developing hypotension or respiratory depression. Gastrointestinal adverse effects associated with opiate use in rabbits include nausea, anorexia, and disruption of gut peri-

Received: 04 Dec 2012. Revision requested: 02 Jan 2013. Accepted: 21 Feb 2013.
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stalsis (ileus).[7,10] Gastrointestinal stasis can become a medical emergency if not detected promptly. Usually, the institution of forced feedings and fluid therapy is sufficient to counteract the reduction in motility that is observed after the administration of an opioid.[14]

A substitute for buprenorphine is a long-acting NSAID such as meloxicam, which is the NSAID used most often for analgesia in rabbits currently.[10,14,30] The popularity of meloxicam is primarily a result of its relative safety, ease of administration, prolonged half-life, and apparent effectiveness.[10] Meloxicam is a cyclooxygenase-2-selective NSAID with antipyretic, analgesic, and antiinflammatory mechanisms of action.[7,14,18] Such agents have fewer side effects than other NSAID that are usually gastrointestinal in origin.[10,12,14,18,30] Meloxicam produces antiinflammatory effects through inhibition of the cyclooxygenase pathway and therefore decreases the production of downstream mediators of inflammation, including arachidonic acid, leukotrienes, and prostaglandins. Meloxicam undergoes extensive hepatic metabolism into nonbiologically active metabolites that are eliminated largely through the kidney. Safety and efficacy studies have not been conducted for NSAID in rabbits.[12,14] Meloxicam pharmacokinetic studies in rabbits have only recently been initiated.[30] Most of our knowledge of these drugs is based on clinical experience and extrapolation of knowledge gained from other species. NSAID are known to affect prostaglandin synthesis in rabbits.[12] Prostaglandins stimulate elimination of soft feces or cecotropes by inhibiting motility of the proximal colon and stimulating motility in the distal colon.[12] NSAID, in general, are contraindicated in animals that are pregnant or have hepatic or renal dysfunction, increased risk of bleeding, hypotension or conditions which may limit organ perfusion or known gastrointestinal ulceration.[12,14,15,18,22]

Many clinical studies involving human patients strongly suggest that total or optimal pain relief cannot be achieved with a single drug or method without significant side effects.[15] Combined analgesic regimens (balanced analgesia) or a multimodal approach to treatment of pain has strongly been recommended.[14] The rationale is that achievement of sufficient analgesia results from additive or synergistic effects between different analgesics,[11,15] with a concomitant reduction of side effects due to using lower doses of analgesics. Although the value of NSAID in minor to severe postoperative pain is well documented, their effect is too small for their use as the sole analgesic in more severe pain states.[6,17,30] However, NSAID do represent an ideal alternative component in the multimodal approach to postoperative pain. The additive or synergistic effects of combining NSAID with systemic opioids is well documented and has demonstrated the ability to reduce the amount of opioid required for adequate analgesia. Studies comparing postsurgical human patients receiving opiates, NSAID, or both report that the combination of opiate with NSAID decreased the occurrence of opioid-related side effects due to a reduction in opioid requirements.[9,11,16,22]

When animals are under stress, glucocorticoids and catecholamines are secreted by the adrenal glands. Traditionally, the concentrations of these hormones in blood have been used to evaluate the physiologic effects of many types of stressors.[28] A problem with this approach is that blood sample collection alone disturbs an animal, increasing its stress level and artificially raising plasma glucocorticoid levels.[25,28] Noninvasive methods for the determination of glucocorticoids or their metabolites are therefore a prerequisite for assessing stress in animals.[1] After being extensively metabolized in the liver, glucocorticoid metabolites subsequently are excreted as conjugates (sulfates or glucuronides) via urine and with bile into the gastrointestinal tract.[25] As a result, cortisol or corticosterone itself is virtually absent in the feces. Thus the use of enzyme immunoassays relying on group-specific antibodies has proven advantageous to measure fecal glucocorticoid metabolites.[21,25] Fecal samples offer the advantage that they can be collected easily without any need to handle the animal. Plasma glucocorticoid levels fluctuate widely as a result of their pulsatile secretion and circadian rhythms.[25,28] Fecal glucocorticoid metabolite levels represent pooled quantities of glucocorticoids that are an aggregation of glucocorticoid metabolites.[25,28]

In the present study, we investigated whether the concurrent administration of buprenorphine and meloxicam potentiated their individual effects sufficiently that their combination provides adequate analgesia when used at amounts recommended in literature as the lowest dose for either drug alone. Measurements of fecal corticosterone metabolites (FCM) after use of the combination were compared with single-drug analgesic regimens in rabbits that experienced a minimally invasive vascular cut-down procedure to gauge the level of physiologic stress.

Materials and Methods

Animals. This study was approved by the Mount Sinai School of Medicine IACUC and is in compliance with the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals*.[2,13]

The study population comprised 39 male New Zealand White rabbits (*Oryctolagus cuniculus*; age, 2 to 3 mo; approximate weight, 3.0 kg) that were obtained from Charles River Laboratories (Wilmington, MA). The rabbits were purchased as part of a study for generation and treatment of atherosclerosis. Vendor surveillance reports indicated that animals were from colonies negative for *Bordatella bronchiseptica*, *Salmonella* spp., cilia-associated respiratory bacillus, *Helicobacter*, *Encephalitozoon cuniculi*, *Pasteurella multocida*, *P. pneumotropica*, *Pseudomonas* spp., and hepatic and intestinal coccidiosis. Rabbits were housed individually in stainless steel cages (62 cm length × 42 cm height × 79 cm depth; Allentown, Allentown, NJ) with perforated floors suspended above a collecting tray. The rabbits were acclimated for 7 d to environmental conditions (room temperature, 20 to 23.8 °C; relative humidity, 30% to 70%), light cycle (12:12-h photoperiod), and commercial rabbit diet (Laboratory Rabbit Diet High Fiber 5326, LabDiet, Purina Mills, Richmond, IN) prior to study initiation. All rabbits had free access to water via bottles.

Experimental design. Rabbits were randomized into 1 of 4 treatment groups after receiving a vascular cut-down procedure of the femoral artery. Rabbits received buprenorphine (0.03 mg/kg SC every 12 h for 3 d; $n = 10$; Buprenex, Renckitt Benckiser Pharmaceuticals, Richmond, VA), meloxicam (0.2 mg/kg SC every 24 h for 3 d; $n = 10$; Metacam, Boehringer Ingelheim, Vetmedia, St Joseph, MO), buprenorphine with meloxicam (0.01 mg/kg and 0.1 mg/kg, respectively, SC every 24 h for 3 d; $n = 10$), or a single dose of 0.5% bupivacaine (0.5 mL; $n = 9$; Hospira, Lake Forest, IL) infused locally at the incision site at the time of surgery. These dosages were based on published recommendations.[12,14]

Rabbits were evaluated clinically throughout the course of the study, and body weights were collected weekly. CBC and serum chemistry analyses were conducted by a contracted laboratory (IDEXX Laboratories, New York, NY) prior to the study to establish baseline parameters and 7 d after the surgical procedure. Rectal swabs were gram-stained at baseline and on day 7 to evaluate for dysbiosis of gastrointestinal microflora.

Rectal swabs were obtained using a sterile, dry swab introduced into the rectum of animals at baseline and on day 7. The swabs were then stained using a commercial Gram stain kit (Cat no. 212539, BD Franklin Lakes NJ) All swabs were examined by the same pathologist (VG) and evaluated qualitatively for the presence of a mixture of microflora that includes small gram-negative bacilli and metachromatic bacilli, which indicates a normal gastrointestinal bacterial population. Swabs were also evaluated for endoparasites.

In the current study, we used a 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassay for quantifying FCM. Details regarding development, biochemical characteristics, and biologic validation of the assay have been described.[24,25] This immunoassay has been validated for and successfully used in rabbits.[8,19,20] Fecal samples were collected in the morning just prior to cage change on days 0, 3, 7, 14, 21, and 28. Cage liners were changed daily, so that each sample represented a pooled fecal sample collected over a 24-h period. Feces were collected from areas of the liner that were free of urine. Rabbits were individually housed, preventing mixing of samples. Approximately the same numbers of pellets were collected from each tray. Fecal samples were stored at –80° C until processing. Each of the individual pooled samples of fecal pellets was processed by manually mixing to ensure a homogenized sample and then adding 0.5 g feces to 5 mL 80% ethanol. The suspension was mixed by using a hand vortexer, centrifuged, separated, and air dried. Detailed descriptions of the assay's protocol have been published elsewhere.[24,25]

The concentrations of the metabolites in the feces were anticipated to reflect many of the factors that influence blood glucocorticoid levels. These include subject- and species-associated differences, circadian rhythm, seasonal variations, sex and age, sensitization, and habituation.[4,24,25,28] Plasma glucocorticoid levels are expected to be higher in male rabbits than in female rabbits, highest in the morning, and higher among rabbits living separately than group housed.[4] For these reasons, rabbits of the same age and sex were selected. Each rabbit was singly housed. Fecal samples were collected in the morning at approximately the same time each day, and each of the rabbits had the same acclimation period to adjust to their environment.

Baseline parameters. Once the rabbits were received from the vendor, they were placed on a standard chow (Laboratory Rabbit Diet High Fiber 5326, LabDiet, Purina Mills) for a 7-d acclimation period, after which the rabbits received a diet containing 4.7% hydrogenated coconut oil and 0.3% cholesterol (Certified Rabbit Chow no. 5322, Purina Mills) for approximately 2 wk. The diet was changed because the rabbits ultimately were used in a study comparing radiographic tracers for assessment of atherosclerotic lesions, and the high-cholesterol diet was necessary to create the plaques. None of the rabbits were imaged or received radiographic dyes during the FCM measurement period. All changes in diet occurred 2 wk prior to the day of the surgical procedure and start of the study (day 0). Baseline parameters were collected prior to surgery. Fecal samples for corticosterone metabolite measurements were collected the morning prior to the procedure. Blood samples for CBC and clinical chemistry analyses were collected from anesthetized animals. Food intake, fecal production, and urine output and Gram stains of rectal swabs were assessed subjectively. Body weight was measured just prior to the surgical procedure. Baseline parameters were recorded as day 0 of the study.

Surgical procedure. In preparation for a routine vascular cut-down, each rabbit was anesthetized with ketamine (30 mg/kg IM; KetaVed, Bioniche Teoranta, Galway, Ireland) combined with xylazine (2 mg/kg IM; AnaSedLloyd Laboratories, Shenandoah, IA). Once sedated, the rabbits were prepared aseptically for surgery and transported to the surgical suite. Oxygen (2 L/min) was provided by face mask to maintain adequate oxygenation. During surgery, respiration rate, heart rate, and oxygenation were monitored via pulse oximetry. Each rabbit underwent a vascular cut-down procedure of the femoral artery as part of a different study. A 4-French Fogarty balloon embolectomy catheter was introduced through an arterotomy and advanced to the descending aorta and withdrawn. The femoral incision and skin were closed in 2 layers with 3-0 Vicryl (Ethicon, Somerville, NJ) for the muscle layer and 3-0 Prolene (Ethicon) for the skin. The rabbits were allowed to recover in their home cages.

The rabbits were monitored by 3 veterinary technicians daily for 10 d after surgery. The observers were not blinded to treatment group because of their extensive involvement in postoperative treatment administration. On days 3, 7, 14, 21, and 28, fecal samples were collected for FCM measurements. On day 7, blood was collected from anesthetized rabbits for a posttreatment CBC count and clinical chemistry analysis. Body weights were measured on days 7, 14, 21, and 28. Gastrointestinal eubiosis was confirmed on day 7. Any rabbit that exhibited either complete lack of appetite or scant to absent fecal production for 3 d was removed from the study and supplemented with metoclopramide and timothy hay.

Data analysis. Dependent variables were FCM levels (measured on days 0, 3, 7, 14, 21, and 28 after surgery) and body weight (measured on days 0, 7, 14, 21, and 28 d postoperatively). Data underwent ANOVA by using SPSS software (version 20.0, IBM, New York, NY), with one between-subjects factor (analgesic condition) and one within-subjects factor (day of data collection). Factors in ANOVA were day (within-subjects) and treatment group (between-subjects). Huynh–Feldt corrected P values are reported to correct significance tests for violations of sphericity. Blood parameters (CBC, chemistries) were collected on days 0 and 7 and analyzed by repeated-measures ANOVA in the same manner. Differences were considered significant when P values were 0.05 or less. Because FCM data were missing for one rabbit in the buprenorphine group on day 14, this rabbit was excluded from analyses that incorporated this data point.

Results

FCM. Prior to surgery, baseline levels of corticosterone metabolites found in rabbit feces were similar among all animals (Figure 1). By day 3 after surgery, rabbits in 3 of the treatment groups (buprenorphine, meloxicam, and bupivacaine) showed increased FCM levels that continued to climb gradually until day 7 and then slowly returned to baseline by day 28. In contrast, in the rabbits given low-dose buprenorphine with meloxicam, FCM was unchanged until day 3, when treatment was discontinued; FCM then began to rise. Peak levels of FCM were similar among the 4 groups, although the buprenorphine–meloxicam group reached peak levels several days after treatment was discontinued, whereas the other groups achieved peak levels around day 3. Repeated-measures ANOVA of FCM measurements across the entire study period (days 0, 3, 7, 14, 21, and 28) revealed significant effects of group ($F_{3, 34} = 3.71$, $P = 0.021$) and day ($F_{5, 170} = 4.576$, $P = 0.006$) and a group × day interaction ($F_{15, 170} = 2.037$, $P = 0.048$). The interaction of group and day appears to be entirely due to a difference in the pattern of data between the buprenorphine–meloxicam group and the other 3 groups (Figure 1); when the buprenorphine–meloxicam group was excluded from the overall analysis, the main effect of day

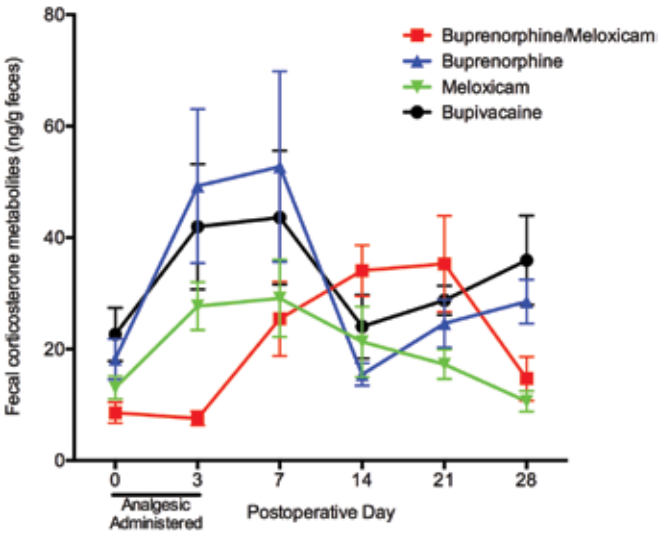

Figure 1. Comparison of fecal corticosterone metabolite levels between treatment groups over time. Analgesics were given on postoperative days 0 through 3, except for bupivacaine, which was given as a single dose on day 0.

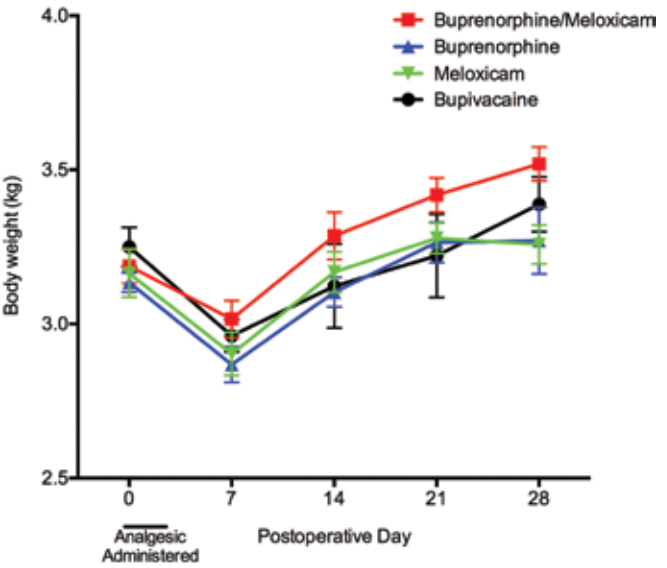

Figure 2. Comparison of body weights between treatment groups over time. Analgesics were given daily on postoperative days 0 through 3, except for bupivacaine, which was given as a single dose on day 0.

remained significant, but the group × day interaction disappeared ($F_{10, 125} = 0.581$, $P = 0.827$). These effects were not present after focused analyses on particular phases of the study. For the period of analgesic administration (days 0 and 3), repeated-measures ANOVA comparing just these 2 d revealed an effect of day ($F_{1, 35} = 17.22$, $P < 0.0005$), a main effect of group ($F_{1, 35} = 4.587$, $P = 0.008$), and a group × day interaction ($F_{3, 35} = 3.041$, $P = 0.042$). In the overall analysis, these effects appear to be driven by the buprenorphine–meloxicam group, whose FCM level did not change between days 0 and 3, whereas the other 3 groups had large increases in FCM. Repeating ANOVA on days 0 and 3 but without the buprenorphine–meloxicam group yielded a main effect of day ($F_{1, 26} = 17.994$, $P < 0.0005$) but no main effects of group or group × day interactions ($F_{2, 26} < 1.292$, $P > 0.29$ for both analyses). FCM on days 0 and 3 did not differ in the buprenorphine–meloxicam group (paired-samples $t_9 = 0.389$, $P = 0.706$). We also analyzed the peak FCM for each rabbit (regardless of day) as the maximal value for each rabbit on any of the days measured; this value did not differ significantly between the treatment groups ($F_{3, 35} = 1.955$, $P = 0.139$). Therefore, although the buprenorphine–meloxicam-treated group did not show the same elevation of FCM at 3 d after surgery as that in the other 3 groups, all 4 groups ultimately reached similar peak levels.

Food intake and fecal and urine output. All 39 rabbits showed decreased food intake on day 1 postoperatively. In general, the quantity of food consumed did not differ among groups. Specifically, pellet consumption from baseline through day 7 did not differ among the treatment groups; after day 7, food consumption gradually began to increase in all treatment groups, returning to baseline levels by day 14. Fecal output declined concomitantly with decreased food intake but returned to baseline by day 14. There were no anorectic rabbits at any point; therefore no treatment with metoclopramide or supplemental feeding was implemented in any of the animals. No rabbits were removed from the study, and urine output remained normal in all treatment groups.

Body weight. All 39 rabbits showed a drop in body weight (about 8%) immediately postoperatively (Figure 2). ANOVA comparing body weight on day 0 with that on day 7 showed a main effect of day ($F_{1, 35} = 113.28$, $P < 0.0005$) that did not differ between treatment groups, with no significant effect of group or group × day interaction ($F_{3, 35} < 1.237$, $P > 0.31$ for both comparisons). The low-dose buprenorphine–meloxicam group showed the least amount of weight loss (approximately 5%) of the 4 groups.

Over the entire 28-d study period, one rabbit in the buprenorphine group was a statistical outlier. On days 0, 7, 14, 21, and 28, this rabbit weighed 3.06, 3.21, 2.85, 2.73, and 2.53 kg, respectively, and thus had lost 0.53 kg relative to its preoperative weight by the end of the study, even though it gained weight during the first postoperative week. Most other rabbits gained weight over the 28-d study period, and no other rabbit lost more than 0.17 kg. When the body weight data were analyzed across the 28-d study period with this outlier case excluded, changes in weight across weeks differed significantly among treatment groups (week: $F_{4, 136} = 74.31$, $P < 0.0005$; week × group interaction: $F_{12, 136} = 2.15$, $P = 0.028$). This interaction appeared to be driven by the greater weight in the buprenorphine–meloxicam group relative to the other groups. This group showed the greatest weight gain by day 28 postsurgically: almost 0.35 kg compared with 0.1 kg in the other groups. Analysis of weight gain (difference in weight on days 0 and 28) revealed a significant effect of group ($F_{3, 34} = 3.497$, $P = 0.026$). Least significant difference post hoc tests indicated significant differences between the buprenorphine–meloxicam group and the bupivacaine-only ($P = 0.004$) and meloxicam-only ($P = 0.022$) groups but not between the buprenorphine–meloxicam group and the buprenorphine group ($P = 0.138$). No other post hoc comparisons were significant.

Hematology and serum biochemistry. CBC and clinical chemistries were evaluated as part of the normal minimal database collected under anesthesia prior to surgery (day 0) and on day 7 after surgery. Parameters of particular interest were WBC count, hematocrit, BUN, creatinine, glucose, ALT, ALP, total protein, and serum electrolytes. At baseline, all rabbits were within normal ranges for all hematologic and biochemical parameters. Although several statistically significant hematologic changes were detected comparing baseline (day 0) and day 7 postoperatively after correction for multiple comparisons, these were extremely small in magnitude and appeared to have no clinical relevance (data not shown). All rabbits remained within normal ranges of reference values despite these minor fluctuations.

Rectal Gram stains. Rectal swabs were used to assess normal microflora and to confirm that gastrointestinal dysbiosis, which might account for abnormal gastrointestinal signs, had not occurred. Based on qualitative assessment of Gram stains, fecal flora remained within normal proportions expected for rabbits within all treatment groups throughout the study (data not shown).

Discussion

The goal of the current study was to determine whether combined administration of buprenorphine and meloxicam, at the lowest dose recommended for each drug as a sole agent, provided adequate postsurgical analgesia without associated complications. To evaluate this hypothesis, FCM was measured to assess physiologic reactions in rabbits after surgery.

Studies of various species of animals have revealed considerable interindividual variation, both in basal and ACTH-induced levels of FCM.[29] Factors that can influence concentrations of immunoreactive FCM include temperature, humidity, and other environmental conditions.[19,28,29] Bacterial enzymes have been reported to increase or decrease levels of FCM if samples were not frozen promptly after elimination.[19,21,29] Contamination with water or urine has been known to affect concentrations of measured steroid metabolites.[24] In addition, high plasma levels of glucocorticoids, but perhaps not mild or moderate levels, may be reflected in concentrations of FCM.[29] Therefore, comprehensive information on the animals' biology and stress physiology must considered when using FCM to assess adrenocortical activity.[29] Collection of samples before and after a known stressful event can be used to evaluate its biologic relevance.

According to FCM levels, combined administration of low-dose buprenorphine and meloxicam appears to mitigate postsurgical stress in rabbits. This assumption was supported by quantifying the amounts of FCM from the rabbits postsurgically at different time points and comparing the postsurgical with presurgical levels. If the individual drugs had a direct effect on glucocorticoid metabolism, we would expect to see that effect in the single dose group FCM levels. Instead, the pattern of FCM levels differs between the single drug groups versus the combination group. The FCM from rabbits in this treatment group remained unchanged for the first 3 d, while the drugs were administered. The meloxicam, buprenorphine, and bupivacaine groups had higher levels of FCM than the combination group during treatment and throughout the study, even though these drugs were used at recommended dosages. Rabbits from the buprenorphine–meloxicam combination group showed the greatest overall weight gain across the 28-d study period.

The increase in FCM after day 3 in the buprenorphine–meloxicam group suggests that the rabbits experienced stress when treatment was discontinued. This finding may suggest that the duration of analgesic administration should be extended beyond 3 d postoperatively, even for minimally invasive procedures like vascular cut-downs. This possibility should be tested empirically, by determining whether prolonged combined administration of buprenorphine and meloxicam (for example, for 7 d instead of 3 d) prevents all postoperative increases in FCM.

The increase in FCM that occurred in the multimodal drug group did not occur at the end of treatment (on day 3) in the other 3 groups; in fact, FCM seemed to be declining. The immediate increase in FCM levels in the single-drug groups may have occurred for several reasons. For example, perhaps none of those rabbits were medicated sufficiently, such that they all had similar responses to surgery. The reduction in FCM levels after day 7 may have occurred solely as a part of healing. The lack of significant differences in the other clinical comparisons (hematology, serum chemistry, and food intake) may indicate that rabbits that appeared to be medicated appropriately according to these metrics were not adequately medicated on the basis of FCM levels. The FCM levels of the multimodal group may have been higher because the rabbits were experiencing pain for the first time after their treatment stopped. Another possible reason for the difference between the multimodal treatment group and the single-drug groups after day 3 is that the multimodal drug regimen may be better at ameliorating acute pain than chronic pain.

The loss of body weight can be explained by the observed decreased food and water consumption but may also reflect postoperative catabolism. The degree and duration to which the values are depressed can be due in part to inadequately controlled postsurgical pain or gastrointestinal disturbances. The rabbits each underwent the same surgical procedure on day 0 and, although vascular cut-down and arterotomy were fairly benign procedures, the procedure required tissue manipulation and compromised blood flow to the distal portion of the extremity. The decreased circulation may cause an unpleasant sensation that was not alleviated by analgesia. Another factor that may have contributed to weight loss across all groups is the anesthetic used. Several reports suggest that weight alterations may be attributed to the physiologic effects caused by certain anesthetics.[6,7] All of the rabbits in the current study received the same anesthetic. Another source of postsurgical weight loss is postoperative catabolism, a normal neuroendocrine response to an injury causing the release of catabolic hormones (catacholamines, pituitary hormones) and suppression of anabolic hormones (insulin, testosterone) and resulting in loss of muscle mass.[31] The responses we observed in our rabbits could have occurred for any of these reasons, independent of the postoperative analgesic used. Although all rabbits in this study lost body weight initially, the buprenorphine–meloxicam group lost the least amount of weight and showed the greatest weight gain by day 28. The rabbits from the 3 single-drug groups had almost twice the amount of weight loss during the 3 d of treatment than did the multimodal group and had the slowest return to baseline weight.

Other traditional parameters that have been used to assess stress in rabbits include hematologic changes, food intake, changes in behavior or activity, and rectal Gram stains. These did not appear to differentiate between the analgesic groups in this study. Many diverse conditions can alter hematologic and biochemical parameters. Parameters of particular interest to us were WBC count and hematocrit, which can reflect infection or chronic stress, indicators of renal function (BUN and creatinine) and liver function (glucose, ALT, and ALP), and serum electrolytes (potassium, calcium, sodium, and phosphorus) and total protein, which assess hydration. All rabbits in each of the analgesic groups remained within normal ranges for all parameters throughout the course of this study.

The cecum is inhabited by diverse microorganisms including protozoa and anaerobic bacteria. In healthy rabbits, high numbers of large anaerobic metachromatic bacteria and protozoa can be seen on Gram stain in feces.[12] Changes in gastrointestinal motility or content of the ingesta that reaches the cecum affect the balance of microorganisms. Changes in circulating glucocorticoid levels can affect the balance of gastrointestinal bacteria. Increased glucocorticoid levels increase coliform counts and alter the aerobic-to-anaerobic bacteria ratio.[12] The rectal Gram stains for the rabbits in the current study remained within

normal limits and helped to confirm that all animals remained clinically normal throughout the course of the study.

Decreases in overall activity and changes in specific behaviors are well documented responses to postsurgical pain in many species.[6,17] This association may be due in part to the stress of surgical procedure and anesthesia, the physical inability to move as a result of the surgical procedure, or a direct result of postoperative pain which may limit movement.[17,31] In our current study, there were no obvious behavioral differences between treatment groups, all of which showed reduction of all active behaviors. The frequency of inactivity decreased over the 7-d postoperative period, suggesting that pain decreased during this time, as expected. Although improved pain control in rabbits should result in less marked reduction of activity, analgesics (especially opioids) can result in sedation, which can contribute to decreased activity.[31]

Part of the study objective was to investigate the consistency of the known side effects of buprenorphine (anorexia, gastrointestinal stasis) in New Zealand white rabbits. Although none of the rabbits treated postoperatively with buprenorphine developed ileus, their median daily food intake and fecal outputs indicated an initial decrease in appetite and perhaps a slight reduction of gastrointestinal motility. However, these rabbits recovered without medical intervention and remained otherwise healthy throughout the study.

Our current study supports the use of FCM as a noninvasive method for assessing postsurgical stress in rabbits and can be used to compare analgesics. We have demonstrated that FCM measurements could differentiate the effect of analgesic regimens after surgery. Specifically, we found that the combined use of low-dose buprenorphine and meloxicam mitigates the FCM response in rabbits better than either of the drugs administered alone and without causing systemic complications. In future studies, we plan to evaluate whether extending the duration of the treatment combination further decreases FCM concentrations overall.

Acknowledgments

First and foremost, we acknowledge the Center for Comparative Medicine and Surgery (CCMS; Mount Sinai School of Medicine) for providing funding for the project. We also thank Ying Dai (Manager, CCMS Comparative Pathology Laboratory) for her meticulous assistance with specimen processing and transport; Ronald Primm, Ignacio Medel, and Pedro Hernandez (CCMS Veterinary Technicians) for their diligent support with the surgical procedure, postoperative care, and collection of the samples; and Mrs Edith Klobeth-Rassam for EIA analysis.

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