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Evaluating Postoperative Analgesics in Mice Using Telemetry

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The study examined the efficacy of preemptive or postoperative analgesia on surgical pain in the mouse. Radiotelemetry transmitters were surgically implanted in 28 female ICR mice. A mock ova implantation surgery was then performed. Mice were treated with a single dose of buprenorphine or flunixin meglumine prior to or after surgery, three doses of buprenorphine, or were untreated. Heart rate, blood pressure, home cage activity, food and water consumption, and body weight were measured. The no-analgesia group showed no significant differences between any parameters collected prior to surgery and those collected at similar times during the day of surgery. Significant increases in mouse activity on the day of surgery occurred with all analgesic treatments, compared with pre-surgical activity. There were no consistent significant changes in any other telemetry parameter after treatment with analgesics compared with no analgesia. Food consumption and body weight the day after surgery were reduced significantly in the animals treated with three doses of buprenorphine compared with untreated mice and mice given a single dose of buprenorphine. We conclude that the mock ova implant procedure does not induce sufficient pain to cause alterations in heart rate and blood pressure in the mouse. Activity was significantly reduced in the first 6 h after surgery in mice without analgesia, compared with activity prior to surgery. There were no significant differences between pre-emptive and postoperative analgesia. Body weight and food and water consumption were poor measures of pain because analgesia alone affected these parameters.

An essential aspect of a quality animal care program is the provision for postoperative analgesia. *The Guide for the Care and Use of Laboratory Animals* states, "The proper use of anesthetics and analgesics in research animals is an ethical and scientific imperative. Fundamental to the relief of pain in animals is the ability to recognize its clinical signs in specific species" (11). However, it is especially difficult to detect pain in mice by typical assessment techniques, and obtaining objective measurements of pain and stress levels in mice has been a considerable and elusive challenge. One study found that 40% of subjective animal pain assessments were inaccurate (3). Alterations in behavior, vocalization (both audible and ultrasonic), postural changes, immobility, and depression all can be expressions of pain (7, 13, 14, 16, 22, 26, 28, 30), however these parameters are often not seen in mice unless severe pain is induced, or they are subtle and are missed without intense observation. Because of the absence of good parameters to detect pain, little is known about post-operative pain in mice and the efficacy of postoperative analgesics.

Relatively new advances in miniaturization led to the development of implantable radiotelemetry units that can be used in the mouse (Data Sciences International, Arden Hills, Minn.). Using these transmitters, we can evaluate physiological changes associated with pain in mice without restraining the animal (1, 2, 6, 15). Pain in animals can be demonstrated by increases in blood pressure, increases in heart rate, alterations in locomotor activity levels, and disruption of normal circadian rhythms (2, 4, 30). Painful stimuli may cause an increase or reduction in activity level depending on whether the animal is nervous or guarding the injury. Therefore, the implantation of radiotelemetry transmitters to examine physiologic changes and activity can be used to study postoperative pain and distress without outside influences. Food and water consumption are also used as a pain assessment technique. Small rodents often show reduced appetite if experiencing pain. This decreased appetite can be detected as a decreased body weight if it is not practical to measure food and water intake for individual animals (8).

In this study we implanted telemetry transmitters in singly housed mice to study postoperative pain. We studied the effects of two analgesic agents on heart rate, blood pressure, activity, food consumption, water consumption, and body weight following a mock ova implant surgery. We hypothesized that preemptive analgesia with buprenorphine or flunixin meglumine would provide improved postoperative pain relief. We also hypothesized that multiple doses of buprenorphine would provide better pain relief than would a single dose.

Materials and Methods

Animals. Twenty-eight adult, female, ICR mice (weight, 30 to 40 g) were obtained from University of Cincinnati Embryonic Stem-Cell Core or directly from Taconic (Germantown, N.Y.). Routine sentinel screenings were performed by Charles River Laboratories (Wilmington, Mass.) to ensure the mice were serologically free of antibodies to cilia-associated respiratory bacillus, *Encephalitozoon cuniculi*, ectromelia, epizootic diarrhea of infant mice virus, Hantaan virus, mouse pneumonitis virus, lympho-

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cytic choriomeningitis virus, lactate dehydrogenase elevating virus, *Mycoplasma pulmonis*, mouse adenovirus 1 and 2, mouse cytomegalovirus, mouse hepatitis virus, mouse parvovirus, mouse thymic virus, minute virus of mice, polyoma virus, pneumonia virus of mice, reovirus type 3, Sendai virus, Theiler's murine encephalomyelitis virus, and *Clostridium piliforme*. The mice were also tested for ecto- and endoparasites. Results were negative throughout the study. Testing for *Helicobacter* sp. was not performed.

The mice were housed individually in nonautoclaved static isolator plastic shoebox cages with corncob bedding (Sani-Chips, P.J. Murphy Products, Montville, N.J.). Mice had ad libitum access to water and pelleted feed (Harlan Teklad 7912, Indianapolis, Ind.). Humidity was maintained between 30% and 70%, and room temperature at 70 ± 2°F (ca. 21 ± 1°C). The light cycle was 12:12 h light:dark. Mice, food, and water were weighed daily 3 days prior to surgery and for 5 days after surgery. Cages were changed 3 days prior to surgery and at the time of surgery during the 8-day monitoring period. The facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, and all activities were approved by the Institutional Animal Care and Use Committee.

Surgical procedures. Mice were anesthetized with isoflurane by using a precision vaporizer with a scavenger system (Forane RM 3 Labmed/Labvac, Anesthesia Equipment Service and Supply, Inc., Elizabeth, Colo.). Sterile technique was used for surgery, including the use of sterile drapes, sterile instruments sterile gloves, a facemask, cap, and clean surgery scrubs or lab coat. For the telemetry surgery, the ventral neck and ventrolateral thorax was shaved, dehaired with a depilatory cream (Sally Hansen Crème Hair Remover, Del Laboratories, Inc., Farmingdale, N.Y.), and disinfected with three alternating scrubs of alcohol and povidone iodine prior to surgery. A 2- to 3-cm midline incision was made from manubrium to lower jaw. A catheter attached to the telemetry transmitter (model TA11PA-C20, Data Sciences Inc., St. Paul, Minn.) was inserted into the left carotid artery, and the transmitter body was tunneled under the skin along the right flank through the same incision. The implant was secured with 7-0 silk ligatures and the incision closed. Mice were allowed to recover for 10 to 33 days after the telemetry implant surgery to allow physiologic parameters to return to normal.

A mock ova implantation surgery was then performed using the same anesthetic and sterile technique. The hair was not shaved, and the area was moistened with alcohol, mimicking the surgery performed by the University of Cincinnati Embryonic Stem-Cell Core. A small 0.5-cm incision was made in the flank, the ovaries isolated and retracted for 30 sec and then replaced in the abdomen. The body wall and skin were closed with 4-0 silk. Surgeries were performed in the early morning (between 7:00 a.m. and 10:00 a.m.) to maintain consistency between the mouse diurnal cycles. The surgeons were both considered to be very skilled at the procedures. The surgery took 10 to 15 min from initiation of anesthesia to completion. Animals were placed on recirculating warm-water heating pads postsurgically until the mice attained sternal recumbency and were moving. The mice typically were ambulatory within 10 min after the completion of the surgery. They were then returned to their cages in the housing room. Some mice had 2 mock ova surgeries performed on them 10 to 16 days apart. Analgesic treatment was randomized to ensure that residual effects were minimized in the study.

Analgesia treatments. Six analgesic regimens were used for the mock ova implant surgery. Mice were treated with either no analgesic (n = 6), 2.0 mg/kg buprenorphine one time before (n = 7) or after (n = 6) surgery, 2.0 mg/kg buprenorphine (Phoenix Pharmaceuticals, St. Joseph, Mo.) before surgery and two more doses at 6-h intervals after surgery (n = 6), or 2.5 mg/kg flunixin meglumine (Reckitt Benckiser Pharmaceutical Inc., Richmond, Va.) once prior to (n = 7) or after (n = 6) surgery. All analgesic agents were given subcutaneously while the mice were anesthetized (5 min prior to surgery or 5 min after surgery). Four additional control groups were used: three to assess the effect of analgesia without surgery (one dose flunixin meglumine [n = 4], one dose of buprenorphine [n = 6], and three doses of buprenorphine [n = 5]), and one to assess the effect of the anesthesia without surgery (n = 4).

Collection of radiotelemetry data. Data for activity level (ACT), heart rate (HR), and blood pressure (BP) from the telemetry transmitters was sampled for 10 sec every 5 min using SILVER SYSTEM Advanced Research Technology hardware and software from Data Sciences International, Inc. The data were collected for 1 to 2 days prior to surgery and then for 5 days after surgery. The Dataquest acquisition system sums up all of the activity counts over a sample period. The activity parameter is generated by the Dataquest acquisition system matrix via changes in signal strength as the animal ambulates horizontally across the receiver. The data are given in counts per min. The activity is not a reflection of a distance that the mouse has traveled but rather of how often the mouse moved during the 10-sec interval.

Collection of body weight and food and water consumption. Mice, food, and water were weighed daily on a scale in the room. All mice were weighed at similar times, and the weighing and activity in the room were limited to the morning. Food and water weight were calculated as the previous days amount minus the remaining amount. This mechanism did not take into account food or water that the mouse removed from the hopper or bottle but did not consume; however it was consistent between all times and mouse groups.

Data analysis and statistics. The telemetry data collected prior to surgery were used to establish baseline parameters for comparing the impact of the surgery and analgesics. Because of the large volume of data collected, the telemetry data were pooled into hourly averages. To better understand the effects of surgery and analgesics, the average telemetry parameters for each hour after surgery until 6 h was compared with the same hours on the day prior to surgery. In addition, the 6-h and the 24-h averages after surgery were compared with the same periods prior to surgery and the days after surgery until 5 days. Furthermore, to minimize between-animal variability, the postoperative data were normalized in light of the corresponding baseline parameters from the day prior to surgery. In addition, food and water consumption and body weight were recorded daily. Similarly, these data also were normalized in light of values from the days prior to surgery.

The data were examined by analysis of variance (ANOVA) followed by Fisher's protected least significance difference test (PLSD) to compare the effects of the different analgesic treatments. Student *t* tests also were performed to compare the effects of each analgesic treatment between the day of and the day

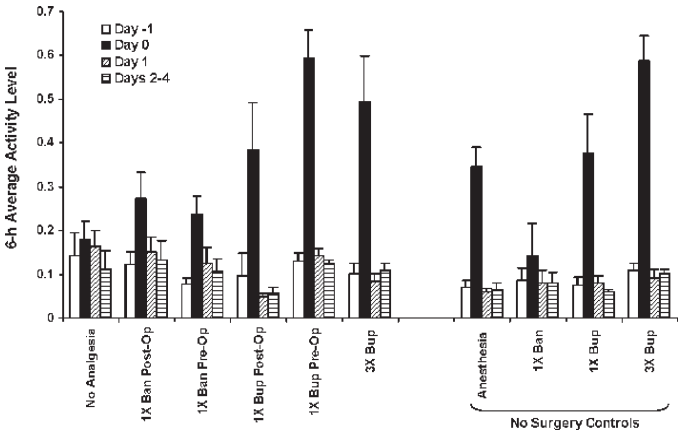

Figure 1. 6-h activity following mock ova implant surgery. Data represent the average of a 6-h interval immediately following the surgery (Day 0), the day after surgery (Day 1), and the next 3 to 5 days (Steady State) compared with the same 6-h intervals from the day before surgery (Day -1). Bars represent the standard error.

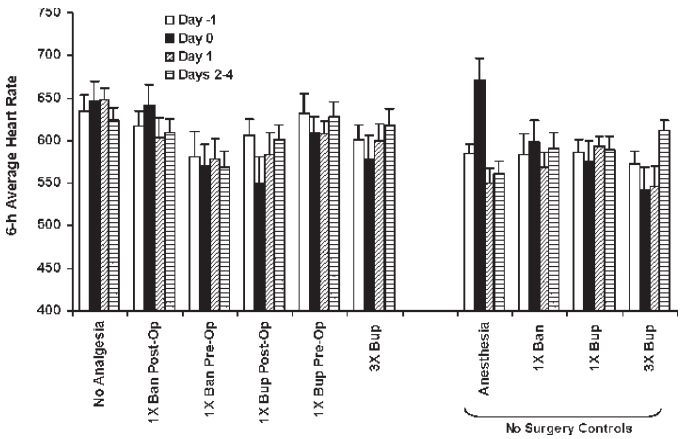

Figure 2. 6-h heart rate following a mock ova implant surgery. Data represent the average of a 6-h interval immediately following the surgery (Day 0), the day after surgery (Day 1), and the next 3-5 days (Steady State) compared with the same 6-h intervals from the day before surgery (Day -1). Bars represent the standard error.

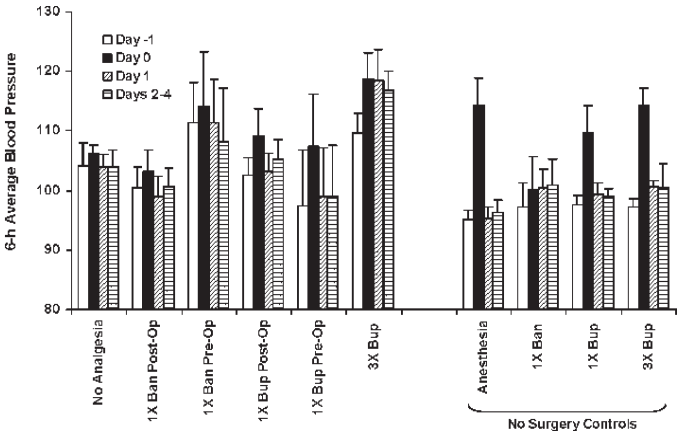

Figure 3. 6-h blood pressure following a mock ova implant surgery. Data represent the average of a 6-h interval immediately following the surgery (Day 0), the day after surgery (Day 1), and the next 3 to 5 days (Steady State) compared with the same 6-h intervals from the day before surgery (Day -1). Bars represent the standard error.

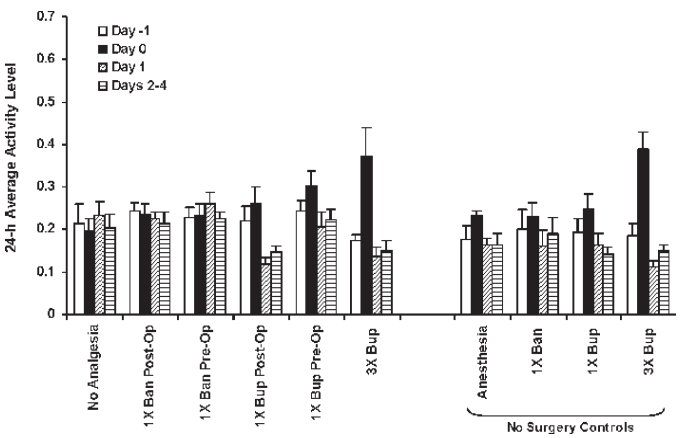

Figure 4. 24-h activity following mock ova implant surgery. Data represent the average of a 24-h interval immediately following the surgery (Day 0), the day after surgery (Day 1), and the next 3 to 5 days (Steady State) compared with the same 24-h intervals from the day before surgery (Day -1). Bars represent the standard error.

prior to surgery. The significance level (α) for all statistical tests was set at 5%. Statistical data analysis was performed using StatView software (SAS Institute, Inc., Cary, N.C.).

Results

No-analgesia group. Six mice had a mock ova implant surgery without analgesia. These animals showed no significant changes in any of the outcome measures at any time point in the pre- and postsurgery parameters. There was no significant change in the body weight, food consumption, or water consumption from the day prior to surgery compared with postoperative data. No significant differences were seen in subsequent days.

Flunixin meglumine treatment. Mice were treated either pre- or postoperatively with 2.5 mg/kg flunixin meglumine subcutaneously. Treatment with a single dose of flunixin meglumine either pre- or postoperatively significantly increased the 6-h post-surgery average ACT level on the day of surgery by one- to twofold compared with values for the day before surgery ($P < 0.05$, Fig. 1). Despite various trends, the single-dose flunixin meglumine treatment did not lead to significant changes in the 6-h post-surgery averages of HR or BP on the day of surgery and the days before and after surgery (Fig. 2 and 3). The 24-h average daily ACT on the day of surgery was comparable with that of the days before and after surgery. Similarly, the 24-h average HR and BP were maintained without significant changes between the day of surgery and the days before and after surgery.

The timing of the drug administration (pre- versus postsurgery) did not influence the changes in the 6-h or 24-h average telemetry data postsurgery (Fig. 1 through 6). Examination of the hourly postsurgery averages revealed significant ($P < 0.05$) differences between the preemptive and postoperative flunixin meglumine treatments. These changes, however, had no pattern and were limited to a few time points (Fig. 7 through 9) and thus were considered to be biologically insignificant.

Comparisons of the flunixin meglumine treatment group with the no-analgesia group showed changes in HR only. There was a decrease in the HR of the group treated preemptively with flunixin meglumine during the second through fifth hour after

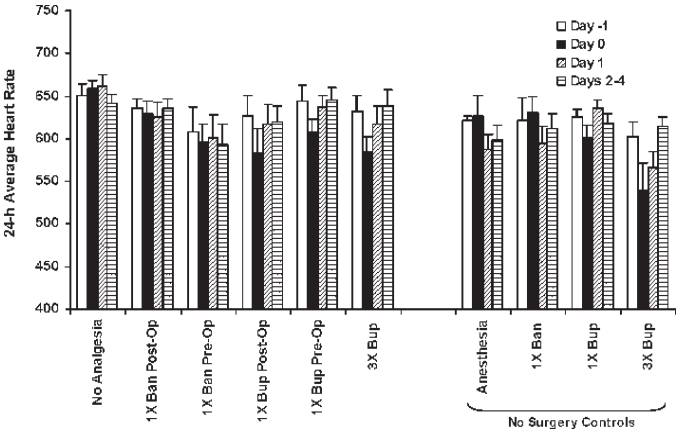

Figure 5. 24-h heart rate following a mock ova implant surgery. Data represent the average of a 24-h interval immediately following the surgery (Day 0), the day after surgery (Day 1), and the next 3 to 5 days (Steady State) compared with the same 24-h intervals from the day before surgery (Day -1). Bars represent the standard error.

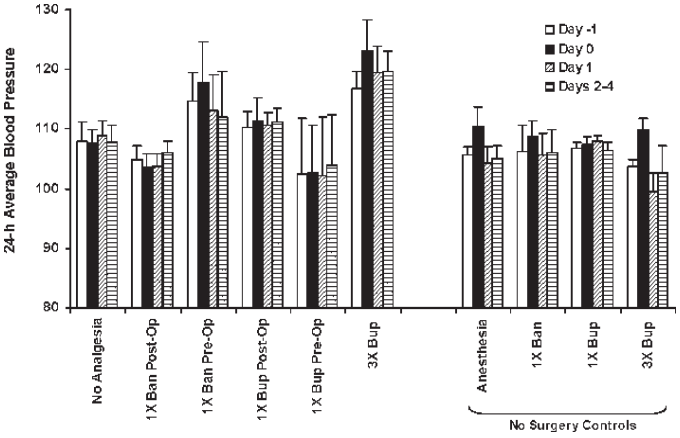

Figure 6. 24-h blood pressure following a mock ova implant surgery. Data represent the average of a 24-h interval immediately following the surgery (Day 0), the day after surgery (Day 1), and the next 3 to 5 days (Steady State) compared with the same 24-h intervals from the day before surgery (Day -1). Bars represent the standard error.

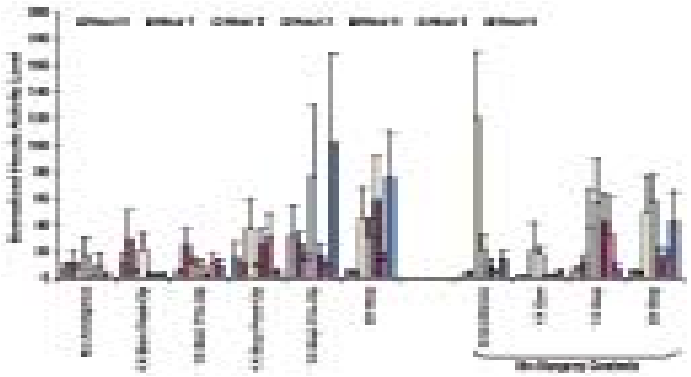

Figure 7. Hourly activity following mock ova implant surgery. Data are normalized by hourly baseline values during the same period on the day prior to surgery or treatment. Hour 0 is the hour just prior to surgery and Hour 1 is the hour immediately after surgery/treatment. Bars represent the standard error.

surgery compared with that of the no-analgesia group. There was a significant decrease in the 6-h and 24-h average HR of the preemptive flunixin meglumine treatment group compared with

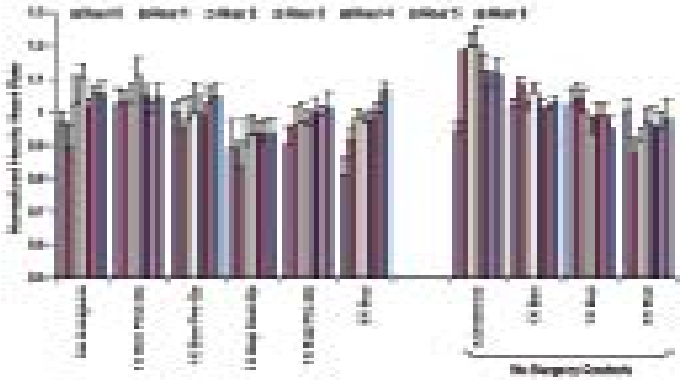

Figure 8. Hourly heart rate following a mock ova implant surgery. Data are normalized by hourly baseline values during the same period on the day prior to surgery or treatment. Hour 0 is the hour just prior to surgery and Hour 1 is the hour immediately after surgery/treatment. Bars represent the standard error.

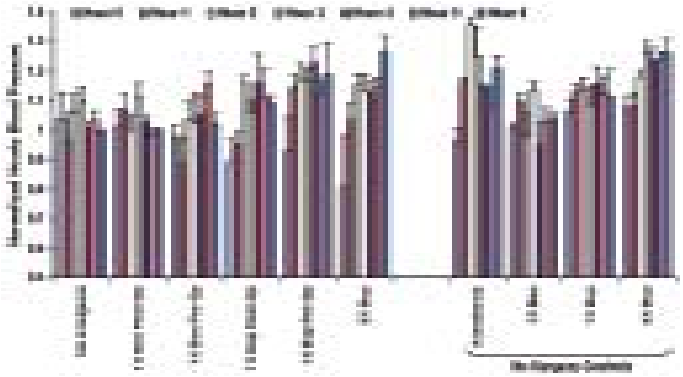

Figure 9. Hourly blood pressure following a mock-ova implant surgery. Data are normalized by hourly baseline values during the same period on the day prior to surgery or treatment. Hour 0 is the hour just prior to surgery and Hour 1 is the hour immediately after surgery/treatment. Bars represent the standard error.

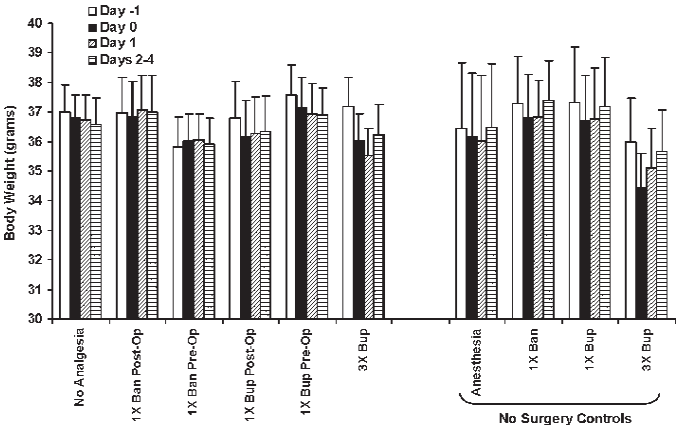

Figure 10. Daily body weight (g). Data represent the body weights recorded every 24 h immediately prior to surgery (Day -1), day after surgery (Day 0), the second day after surgery (Day 1), and the next 3 to 5 days (Steady State). Bars represent the standard error.

the no-analgesia group.

Neither the preemptive nor postoperative flunixin meglumine treatment induced any changes in food or water consumption or body weight before and after surgery (Fig. 10 through 12).

Buprenorphine treatment. Mice were either treated with a

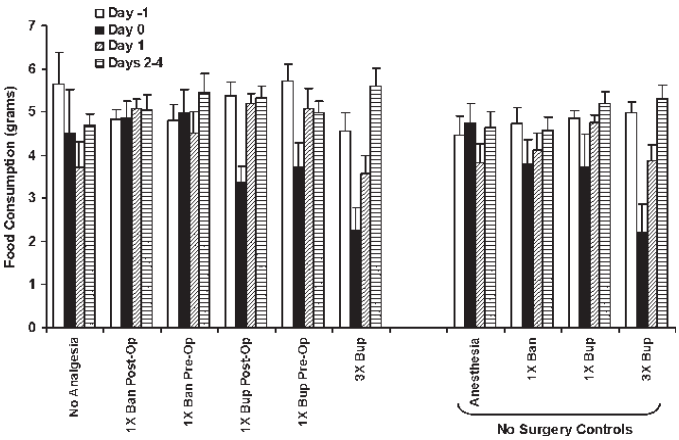

Figure 11. Daily weight (g) of food consumed. Data represent the food consumption recorded every 24 h immediately prior to surgery (Day -1), day after surgery (Day 0), the second day after surgery (Day 1), and the next 3 to 5 days (Steady State). Bars represent the standard error.

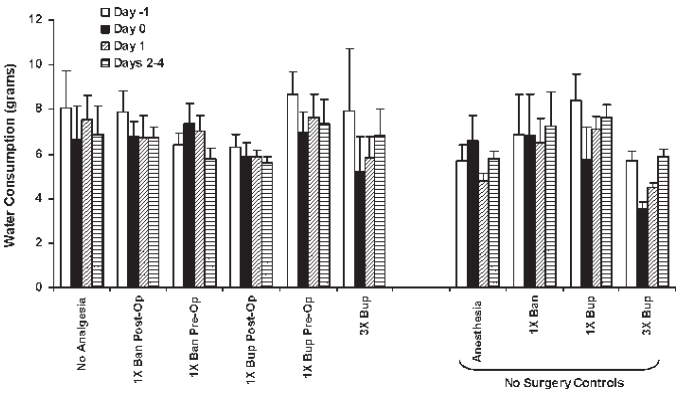

Figure 12. Daily weight (g) of water consumed. Data represent the water consumption recorded every 24 h immediately prior to surgery (Day -1), day after surgery (Day 0), the second day after surgery (Day 1), and the next 3 to 5 days (Steady State). Bars represent the standard error.

single preoperative or postoperative dose of 2.0 mg/kg buprenorphine subcutaneously or with three doses 6 h apart.

Significant ($P < 0.05$) changes were observed when examining the mice treated with buprenorphine with their baseline data from the previous day. The treatment with all dosages of buprenorphine significantly increased the average ACT in the 6 h following the mock surgery compared with the same 6-h period on the day prior to surgery by four- to fivefold (Fig. 1). All treatments with buprenorphine led to a reduction in the 6-h average HR following surgery, albeit these reductions were not statistically significant (Fig. 2). By contrast, the treatment with a single or three doses of buprenorphine significantly increased the 6-h average BP following surgery by 6% to 10% compared to the day before surgery ($P < 0.05$; Fig. 3).

Furthermore, there was a significant ($P < 0.05$) increase in the 6-h average ACT of the group treated with a single dose preoperative buprenorphine treatment compared with that of the no-analgesia group (Fig. 1). There were significant ($P < 0.05$) reductions in the 6- and 24-h average HR of the single dosage postoperative treatment group compared with the no-analgesia group (Fig. 2 and 5). In addition, there was a significant ($P < 0.05$) increase in the 24-h average BP of the group treated with three doses of buprenorphine compared with that of the no-analgesia group (Fig. 6). There were no significant differences when comparing single dose preemptive buprenorphine administration with postoperative buprenorphine administration in the 6- and 24-h average telemetry data.

Examination of the hourly postsurgery averages revealed significant ($P < 0.05$) differences between the preemptive and postoperative buprenorphine treatments. Similar to the results for the flunixin meglumine treatment, the differences had no pattern and were limited to a few time points (Fig. 7 through 9), and thus were considered to be biologically insignificant.

There was also a significant ($P < 0.05$) reduction in body weight and food consumption on the surgery day in all mice treated with buprenorphine (Fig. 10 and 11). The three-dose buprenorphine treatment mice had the greatest reduction in body weight and food consumption.

Anesthesia and analgesia without surgery. As control groups, we treated mice with buprenorphine or flunixin meglumine or anesthetized them without performing surgeries to determine the effects of these treatments in the absence of the pain inducing surgeries. The mice that were anesthetized without surgery had significant ($P < 0.05$) increases in average ACT (Fig. 1) and BP (Fig. 3) during the 6-h period following treatment compared with the same period on the previous day. Mice that were anesthetized without surgery also had a significant ($P < 0.05$) increase in average HR during the 6-h period following anesthesia compared with the same period on the previous day (Fig. 2).

The treatment with flunixin meglumine without surgery resulted in no significant changes in the 6-h post-treatment average ACT level, HR, or BP on the day of treatment compared to the previous day. The treatment with a single or three doses of buprenorphine without actually performing the mock surgery significantly ($P < 0.05$) increased the average ACT and BP and decreased HR in the 6 h following the treatment compared with the same 6-h period on the day prior to treatment.

In addition, the treatment with three doses of buprenorphine without performing the mock surgery significantly ($P < 0.05$) reduced food and water consumption and consequently body weight on the day of treatment compared with those of the previous day. No other control treatments without surgery (anesthesia, single dose of flunixin meglumine, single dose of buprenorphine) had any significant effects of food and water consumption and body weight (Fig. 10 through 12).

Discussion

Mock ova implant surgeries were performed via flank laparotomy in adult female mice. The effects of different analgesics—flunixin meglumine (Banamine) and buprenorphine—given prior to or after surgery as single or three doses on the postsurgery telemetry parameters including ACT, HR, and BP were monitored and compared with baseline values established preoperatively. For controls, mice not treated with any analgesics underwent the mock ova implant surgery. Additional controls included mice treated with the same analgesic regimens or anesthetized only (without actually undergoing surgery).

It is well documented that physiological responses to a noxious stimulus include elevations in both HR and BP (10, 21, 23, 25). The response to an acute episode of visceral pain such as distension of the duodenum of rats is an elevation in HR and mean arterial BP (23). Chronic abdominal pain of visceral origin has

also been shown to cause an increased resting heart rate in humans. Examples of this situation include chronic conditions such as irritable bowel syndrome and interstitial cystitis, for both of which the origin of the pain or noxious stimuli is visceral (10, 21). Horses suffering from chronic laminitis, in which the source of pain is peripheral, also have been shown to have an elevated mean resting HR (25).

Surprisingly, there were no significant alterations in HR or BP in mice not given any analgesia. There are two plausible interpretations of this result. First, unlike other species, ICR mice may not respond to pain with an elevation of HR or BP. Alternatively, the pain induced by a mock ova implant surgery is either below the threshold required to cause alterations in HR and BP or is not be the right type of pain to induce changes in HR and BP. We were not able to determine which of these interpretations is correct. However, the absence of differences in activity, body weight, food and water consumption in the no-analgesia group before and after surgery supports the contention that the mock ova implant surgery did not cause debilitating pain. The one caveat to that hypothesis is the lack of an increase in ACT in the untreated mice. Mice are in the sleep phase of their diurnal cycle during the day. Because the mice were awakened for the surgical procedure, we would expect that they would be more active after the surgery compared with their ACT on the previous day. This hypothesis was true in all analgesic-treated mice, as there was an increase in ACT compared with that the day prior to surgery. Interestingly, mice that were anesthetized and had no surgery also had a significant increase in ACT following the procedure. This finding may suggest that the surgical procedure led to a reduction in ACT that went unrelieved in mice not treated with an analgesic. We speculate that mice experiencing pain may be more likely to remain still. This theory would explain the reduced activity in the mice that did not receive analgesics. However, this is not clear-cut, because the increased ACT seen in the analgesia-treated groups could merely be a side effect of the analgesic treatment, as mice given an analgesic in the absence of the surgery also had increased activity. Therefore, it is possible that the increased activity is a side effect of the administration of the analgesic that may not reflect pain relief. In addition, even further complicating the interpretation of the results is that animals that have colic typically show increased ACT because of pain. Further examination of the physiologic response of mice to pain is necessary to determine the effects of the analgesic agents on the postoperative activity of the mouse.

Because of the absence of a significant effect from the surgery itself on the mice without analgesia, interpretation of our two hypotheses is confounded. The first hypothesis examined whether preemptive analgesia is better than post-surgical analgesic administration. Preemptive analgesia implies that the analgesic agent is given before the painful stimulus. The concept of preemptive analgesia is based on findings that intense activation of nociceptive primary afferent fibers by tissue injury and inflammation produces central sensitization or hyperexcitability of nociceptive neurons in the spinal cord dorsal horn (5, 31). Preventing initial nociceptive afferent input to the spinal cord prevents the development of sensitization and thereby reduces postinjury pain and hyperalgesia. For this reason, preemptive analgesia has been advocated and used prior to a number of surgical procedures to reduce and manage postoperative pain (32). Despite the scientific basis for the use of preemptive analgesia, the results of such treatment remain controversial. Numerous studies in humans show either improvement or no difference when preemptive analgesia is used, and results of their value is still questioned (19). Kelly concluded that the most important condition for effective preemptive analgesia is the establishment of an effective level of antinociception before injury and continuing the effective analgesia into the inflammatory phase of healing (19).

In our studies no significant differences between the preemptive and postoperative treatments were observed consistently and likely were masked by animal-to-animal variation. One noticeable improvement included the reduction in the HR in the flunixin meglumine preemptive treatment group. However, no other results showed a benefit to preemptive analgesia with flunixin meglumine or buprenorphine for mock ova implant surgery. It is conceivable that the length of time between the analgesic administration and the surgery (painful stimulus) may influence whether preemptive treatment produces an improved pain relief effect. In our study, the analgesic was given only 5 min prior to surgery so that there was a limited time for the agent to reach peak efficacy. We chose to do so in order to balance the requirements for preemptive analgesia of the mouse with compliance of use by investigators using mice in various studies. If an analgesic can be administered during the surgery setup, it would receive greater compliance than necessitating treatment 30 to 120 min prior to the procedure. Therefore, because the analgesics were given immediately prior to surgery, and only approximately 10 min before the anesthesia wore off, we likely did not achieve optimum analgesia by completion of the procedure and differences in the time of onset of both pre- and postoperative treatments may have been negligible. Therefore, the intended prevention of the initial central sensitization in the preemptive analgesic administration may not have been accomplished because of the limited lead time prior to surgery. Future studies must examine earlier administration of analgesics to determine the efficacy of preemptive analgesia and perhaps use a more severe surgical intervention to ensure that prolonged pain is present.

The second hypothesis was that multiple doses of buprenorphine would improve pain relief after surgery. We examined whether multiple doses of buprenorphine given at 6-h intervals would provide prolonged and improved postoperative pain relief compared with a single dose of buprenorphine. We found no significant changes in the telemetry parameters due to treatment with three doses of buprenorphine. However, because of decreased food consumption that resulted in significant weight loss, it is our conclusion that three doses of buprenorphine resulted in physiologic alterations that adversely affected postoperative recovery. In light of the postoperative changes in HR, BP, and ACT, we believe that the effects of the anesthetic event and surgery (with or without analgesia) were resolved by 6 h postsurgery. Therefore, it is likely that the additional treatment with buprenorphine at 6 and 12 h postsurgery led to further distress to the mice because of the extra handling for the injection and by prolonging inappetance.

Buprenorphine (Buprenex) is a partial opiate agonist with dose-related analgesic properties. Effective dose and duration of effect are not well documented in mice. Recommended dosages of buprenorphine in the mouse range from 0.008 to 2.5 mg/kg (12, 20, 24). This broad range of recommended doses demonstrates

the basic poor understanding of the efficacy of this agent. In addition, clinical responses suggest that the duration of efficacy of buprenorphine in mice is from 8 to 12 h (8). However, data in response to hot plate and tail flick tests show that buprenorphine analgesia (2 mg/kg) is effective for only 3 to 5 h in mice (9). This large variation in reported duration of effect and dosage of buprenorphine further demonstrates our inability to easily identify pain in rodents. To ensure efficacy, we selected a dose on the high side of the broad range of recommended dosages with an intermediate duration (9, 12, 20, 24). This high dose likely contributed to the inappetance in the mice. Although three doses may have been too much for this type of surgery, additional doses may be beneficial in more severe surgical procedures. A one-time intervention with analgesic without subsequent doses during the postoperative period may just delay the onset of nociception and central sensitization, thereby defeating the purpose of preemptive analgesia.

Difficulty in interpreting the results of this and previous studies can be attributed in great part to failure to clearly identify signs of pain in mice. The first key priority in future experiments should be given to developing unambiguous methods for pain detection. Different measurement techniques to determine whether mice are experiencing postoperative pain are being sought and investigated. Karas and colleagues have been studying postoperative behavior and weight changes in mice following either a laparotomy or thoracotomy to identify signs of pain. In their studies, singly housed mice in standard shoebox cages are videotaped for 5-min intervals every 3 h for 48 h. Using a camera that produces images during the mouse's active period (darkness), they demonstrated that typical mouse behaviors such as reaching for the top of the cage, climbing on the roof of the cage, and eating and drinking were attenuated markedly following surgery compared with those of control groups that have had anesthesia only (17, 18). In addition, they demonstrate that mice undergoing surgery spend significantly more time sleeping and experience weight loss of 8 to 12% over the initial 24 h following surgery. Other behaviorally based pain scoring methods are being developed. One such method involves observation of changes in behavioral activities in the rat (27). In these studies, seven behavioral activities were monitored following laparotomy in Fischer rats, and the researchers identified behaviors such as back arching, fall/staggers, writhing, and poor gait as indicators of pain. In this model, carprofen and high-dose meloxicam were found to reduce the number of altered behavioral activities indicative of pain compared with those of no analgesic or low-dose meloxicam. This finding provides evidence that carprofen and high-dose meloxicam reduce postoperative pain. Therefore, it is conceivable that behavioral changes may reflect pain better than do physiologic parameters such as HR and BP.

Still other groups are examining the effects of surgical pain on vocalization in rats (29). These studies look at alterations in vocalization patterns in rodents after a painful procedure. The sensitivity of vocalization technique for postoperative monitoring is not known. Although numerous models for detecting postoperative pain in mice and rats are in development, at the time of this writing there is no proven method available. The results of this current study further demonstrate the need for adequate postoperative analgesia in rodents.

Several conclusions can be made from our study. First, no significant differences were observed between preemptive and postoperative analgesia. Second, multiple doses of buprenorphine were detrimental in that they caused increased weight loss following surgery. Third, unlike in other mammals, BP and HR may be poor indicators of pain or pain relief in mice because the analgesic agents may have side effects on these two parameters independent of the presence of a painful stimulus. Finally, food and water consumption and body weight were not accurate indicators of pain. Declines in these parameters were seen in response to the analgesic alone, even in the absence of the surgical procedure.

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