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Authors

Farkas, Michelle

Dorn, Shanelle

Muller, Liam

et al.

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Pharmacokinetics, Fecal Output, and Grimace Scores in Rabbits Given Long-acting Buprenorphine or Fentanyl for Postsurgical Analgesia

Michelle R Farkas, DVM,[1,*] Shanelle Dorn, BS,[1] Liam Muller, PhD,[2] Vikram Pal Singh, PhD,[3] Yadira J Sepulveda, MS,[4] Raymond T Suhandynata, PhD, DABCC,[4,5] Jeremiah D Momper, PharmD, PhD,[4] Koichi Masuda, MD,[6] and Philip J Richter, DVM, PhD[1]

The New Zealand white rabbit (*Oryctolagus cuniculus*) is a frequently used surgical model. Pain management after surgery is a critical aspect of animal welfare. Recently, a long-acting buprenorphine formulation (Ethiqa XR; EXR) was approved for use in rats and mice but has not yet been investigated in rabbits. The current study aimed to determine whether a single subcutaneous dose of 0.15 mg/kg of EXR could achieve and maintain therapeutic buprenorphine plasma concentrations (0.1 ng/mL) for 72 h in male and female rabbits. We also evaluated the safety profiles of EXR and the fentanyl patch (FP) by assessing fecal output after surgery, because opioids are known to decrease intestinal motility. Behavior and pain scores were compared for rabbits that received either EXR or the FP after undergoing an annulus puncture procedure to induce osteoarthritis. EXR at 0.15 mg/kg SC provided a shorter time to onset and sustained analgesia for 72 h in male and female rabbits, whereas the FP provided suboptimal analgesia after 48 h. Both EXR and FP reduced fecal output after surgery. Output returned to baseline levels within 72 h for the EXR group and remained slightly below baseline at 96 h after surgery for the fentanyl group. Grimace pain scores revealed no significant difference between treatment groups. These results suggest that EXR is a safe and effective option for postoperative pain management in rabbits.

Abbreviations and Acronyms: EXR, Ethiqa XR; FP, Fentanyl Patch; NZW, New Zealand White Rabbit

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Introduction

The New Zealand white (NZW) rabbit (*Oryctolagus cuniculus*) is a common model for ophthalmology, orthopedic, cardiovascular, and neurologic research in which the rabbit may undergo major and minor surgical procedures.[2,14,16,17,20] Postoperative pain management is an important consideration for veterinarians.[3,8] In rabbit orthopedic surgical models, providing adequate postoperative analgesia can hinder the natural degeneration process that is required to create a representative model of orthopedic disease. For example, nonsteroidal antiinflammatory medications such as flunixin meglumine and meloxicam are implicated in impairing bone healing by interfering with prostaglandins and osteoblast proliferation and by inhibiting inflammation.[5] As a result, these agents are frequently contraindicated.[11,14,16,24] Therefore, opioids tend to be the analgesic of choice for postoperative pain management in osteoarthritis and degenerative disc disease rabbit models.[16]

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[1]Animal Care Program, University of California San Diego, La Jolla, California; [2]Department of Molecular Biology, University of San Diego, La Jolla, California [3]Department of Psychology, University of California San Diego, La Jolla, California; [4]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, California; [5]Department of Pathology, University of San Diego, La Jolla, California; and [6]Department of Orthopaedic Surgery, University of California San Diego, La Jolla, California
**Corresponding author. Email: m2farkas@ucsd.edu*

Fentanyl is a schedule II μ opiate agonist that is commonly administered to rabbits by using a transdermal patch. Transdermal fentanyl has been shown to provide 72 h of pain relief in rabbits when applied directly to the skin on the neck.[9,18] Prior pharmacokinetic studies have suggested that the ideal analgesic range for fentanyl is 0.5 to 2.0 ng/mL in humans.[9,18] This range is also considered analgesic in the rabbit.[9,18] The fentanyl patch (FP) must be applied to the skin 12 to 24 h before surgery. Factors such as the amount of subcutaneous fat, integrity of the skin, body temperature, and arrangement of the hair follicles may contribute to variability in the systemic absorption of fentanyl.[18] Furthermore, FP are generally fixed to skin with tissue glue and can fall off, resulting in further variability in the absorption of the drug and creating a foreign-body ingestion risk for the rabbit.[18] Adverse effects to fentanyl include moderate sedation or respiratory depression.[9,18]

Another analgesic option for rabbits is buprenorphine, a schedule III partial μ agonist and κ and γ antagonist. Typically, buprenorphine is administered via subcutaneous or intravenous injection. Buprenorphine is considered analgesic in the rabbit at concentrations of 0.1 ng/mL based on current literature.[1,5] Adverse effects of buprenorphine in rabbits include inappetence, decreased gastric motility and fecal output, and tissue granuloma reactions at the injection site.[4] Despite these adverse effects, many studies show that buprenorphine is safe and well tolerated in rabbits.[1,8]

The standard dose range of buprenorphine HCL for rabbits is 0.01 to 0.05 mg/kg administered as an intravenous, intramuscular, or subcutaneous injection every 6 to 12 h. Because of the need for frequent redosing with standard buprenorphine HCL, many long-acting formulations of buprenorphine have been developed for use in animals. Some studies indicate that excessive handling can be an unnecessary stressor for rabbits; long-acting analgesic formulations could reduce the necessary frequency of handling.[1,8,17,20,21] The long-acting formulations include an extended-release buprenorphine polymeric formulation (buprenorphine SR) and a high-concentration formulation that is FDA labeled for subcutaneous use in cats (Simbadol). Previous studies have shown that high concentrations of buprenorphine are associated with neurologic signs such as depression, horizontal nystagmus, circling, and ataxia in rabbits, which limits use of the high-concentration formulation as an option for long-acting analgesia in rabbits.[1] Furthermore, variation in detectable plasma drug levels reported in prior studies provides evidence that Simbadol may not provide adequate analgesia in rabbits.[21] Prior studies have found buprenorphine SR to be associated with subcutaneous tissue reactions in mice and rats.[12,13,23] In addition, because this drug is available only through veterinary procurement, obtaining it for research use can be complicated.

Ethiqa XR (EXR) is an injectable suspension of extended-release buprenorphine that has been FDA-approved for the control of postprocedural pain in mice and rats. EXR contains a lipid-bound buprenorphine hydrochloride suspended in a medium-chain fatty acid triglyceride oil.[12,13] A previous pharmacokinetic and behavioral study of EXR reported that this drug provides analgesic plasma levels in marmosets.[7] However, EXR has not yet been evaluated in rabbits.

The goal of the current study was to compare the pharmacokinetics, safety, tolerability, and efficacy of EXR and FP in NZW rabbits (*Oryctolagus cuniculus*). We hypothesize that the EXR will provide the following benefits to rabbits: 72 h of analgesia, minimal effects on gastrointestinal motility, and improved pain scores as compared with FP.

Materials and Methods

Animals. A total of 20 (10 male, 10 female) NZW rabbits (*Oryctolagus cuniculus*) were obtained from 2 sources: Charles River Laboratories (Wilmington, MA) and Western Oregon Rabbit (Philomath, OR). They ranged between 5 and 7 mo old, and their weights ranged between 4 and 6 kg.

Prior to their arrival at the facility, each Charles River Laboratories rabbit (10 males) were tested by the vendor and determined to be free of reovirus, *Bordetella bronchiseptica*, *Helicobacter* spp., *Lawsonia* spp., *Pasteurella* spp., *Salmonella* spp., *Treponema* spp., Tyzzer disease pathogens, CAR bacillus, *Cheyletiella parasitovorax*, *Leporacarus gibbus*, *Psoroptes cuniculi*, other ectoparasites, *Passalurus ambiguous*, other helminths, *Eimeria* spp., *Eimeria stiedae*, other intestinal protozoa, and *Encephalitozoon cuniculi.* Prior results from the Charles River Laboratories vendor revealed that rabbits housed in the same room were historically positive for *Pasteurella aeruginosa*; however, no respiratory signs, nasal discharge, or subcutaneous abscesses were noted in the rabbits enrolled in this study. Each Western Oregon Rabbit rabbit (10 females) were negative for *Encephalitozoon cuniculi*, *Treponema cuniculi*, *Clostridium piliforme*, *Pasteurella multocida*, *Salmonella* spp., and hepatic coccidia based on vendor tests.

A complete physical exam was performed on each rabbit by the Animal Care Program veterinarians upon arrival to the vivarium. All rabbits were allowed to acclimate for 14 d before the start of the study. Rabbits were housed individually in either metal or plastic pan rabbit racks (Euro Rabbit; Allentown Caging Equipment, Allentown, NJ) in an AAALAC-accredited facility with a 12:12 h light:dark cycle (on at 0600 PST; off at 1800 PST), temperature of 65 ± 2 °F (18.3 ± 2 °C), and average humidity of 46% (range, 20% to 72%). Rabbits were fed a commercial pelleted diet (Teklan Global High Fiber Rabbit Diet; Envigo, Madison, WI) and either reverse osmosis or softened tap water in glass water bottles ad libitum. Supplements included Critical Care Herbivore Apple-Banana (Oxbow Animal Health, Omaha, NE), which was provided for the first 3 d after surgery as well as timothy hay cubes (LabDiet, Brentwood, MO), carrots, and apples. All animal work conducted in this study was approved by the IACUC and was performed at an AAALAC-accredited facility.

Surgery. Rabbits were randomly divided into 4 experimental groups (n = 5 in each group): EXR male, EXR female, FP male, and FP female. Each rabbit served as its own control because its individual preoperative behavior could be compared with its postoperative behavior.

For the FP group, baseline blood samples were collected and the FP placed at 1900 PST on the evening before surgery. For placement of the 12 µg FP, the hair was shaved between the shoulder blades, and the skin was cleaned with an alcohol wipe. Skin glue was applied to each corner of the patch and the patch was firmly pressed into the skin between the shoulder blades.

For the EXR group, baseline blood samples were collected on the morning before surgery. A 22-gauge needle and 1-mL syringe were used to inject EXR 0.15 mg/kg SC during anesthesia induction. This dose was determined based on consultation with the manufacturer of EXR. Each rabbit was sedated with ketamine hydrochloride (35 mg/kg; Dechra, Overland Park, KS) and xylazine (5 mg/kg SC, VetOne; MWI Animal Health, Boise, ID). In addition, rabbits received cefazolin (22 mg/kg SC; Hikma Farmaceutica, Terrugem, Portugal) and 50 mL SC lactated Ringer's solution (ICU Medical, Lake Forest, IL). After administration of the ketamine and xylazine sedation, all rabbits were placed on a nosecone with 1% to 5% isoflurane. Once at an adequate plane of anesthesia, all rabbits were intubated. Anesthesia was maintained with isoflurane 1% to 5%.

The surgical procedure was as follows: A 6- to 7-cm incision was made through the skin and epaxial muscles cranial to the anterior spinous iliac crest to expose the anterior surfaces of 3 consecutive intervertebral discs. The level of dissection was confirmed by placing a titanium clip and performing an interoperative radiograph. An 18-gauge needle with a 5-mm restriction sleeve placed on the end was used to puncture the disc in the lumbar spine at the level of L2/3 and L4/5. The needle passed through the anterolateral part of the anulus fibrosus into the nucleus pulposus and was held there for 5 s to induce the injury. The wound was closed in layers. All rabbits recovered uneventfully and were returned to their home cages once they regained sternal recumbency. After this study was complete, all rabbits were used in a subsequent study to assess the effectiveness of a novel compound in improving joint degeneration associated with osteoarthritis.

Behavioral observation. To obtain baseline grimace scores, 2 cameras (OpenMV Cam H7; OpenMV, Atlanta, GA) were placed on the cage door of each rabbit enclosure 2 d before surgery. Python 3 (Version 3.9; Python Software Foundation, Wilmington, DE) was used to program each camera to record for 15 min once a day at 1100 PST. Recordings were obtained on each of the 2 d before surgery, surgery day and 3 -d postoperatively. A total of 3 videos obtained on surgery day from 2 EXR females and one

Table 1. Grimace score parameters used for behavioral observation

Parameter	0	1	2
Eye appearance	Eyes open	Eyes slightly closed (squinting)	Eyes closed completely
Ear position	Ears upright	Ears partially lowered at a 45° angle or rotated to the side	Ears pointing back
Movement in cage during the 15 min observation period	Very active, moved 15 or more times	Moderately active, moved fewer than 5 times	No movement, remained motionless

Total scores range from 0 to 6, with a higher score indicating a state that was more consistent with pain in rabbits.

EXR male rabbit were removed from analysis because of poor video quality. In addition, one video obtained on preoperative day 2 from an EXR male rabbit before surgery was removed from the analysis because of poor video quality. Six observers who were blind to the treatment group reviewed each video after the completion of the study and assessed the following parameters: ear position, orbital tightening, and motion. Rabbits were given a score between 0 and 2 for each parameter (Table 1). The mean of the 6 observer scores for each individual rabbit at each time point was used for analysis, with a higher score indicating more pain or distress. Each rabbit's score was compared with its own individual baseline score in the assessment. This scale is a novel approach to behavioral observation in the rabbit.

Blood collection. Blood collection was performed at the following time points for all rabbits given EXR: 0 (predose), 1, 4, 8, 12, 48, and 72 h after administration. For rabbits that received FP, blood was collected at the following time points: 0 (predose), and 4, 8, 24, 48, and 72 h after surgery. All rabbits were anesthetized with 2% to 5% isoflurane administered through a face mask, and approximately 3 mL of blood was obtained at each time point from the central ear artery by using a 23-gauge butterfly needle attached to a 3-mL luer-lock syringe. All blood samples were collected into an EDTA tube and centrifuged at 3,000 × *g* for 10 min (Eppendorf Biotech, Hamburg, Germany). Plasma obtained from each sample was stored in a −80 °C (−112 °F) freezer until analysis. All samples were analyzed for either buprenorphine or fentanyl concentrations using validated liquid chromatography tandem mass spectrometry (LC-MS/MS) assays, as described below.

Determination of plasma fentanyl concentration by ultra-high-performance liquid chromatography tandem mass spectrometry. Calibrators and quality control (QC) samples were prepared from certified reference material: fentanyl (Cerilliant catalog#: F-013). Internal standard (IS) was prepared from certified reference material: fentanyl-d5 (Cerilliant catalog#: F-001). Working stocks of calibration standards, QCs, and internal standards were prepared in methanol before spiking into plasma. An external calibration curve using linear regression to plot the peak area ratio (calibrator: IS) compared with concentration with 1/× weighting resulted in a linear curve, R2 30.99, over the full analytically measurable range (AMR). The AMR for the fentanyl assay was 0.75 to 100 ng/mL.

Ultra-high-performance liquid chromatography (UHPLC) tandem mass spectrometry (MS/MS) was performed in multiple reaction monitoring (MRM) mode on an Agilent Infinity II 1290 and SCIEX 6500+ QTRAO triple quadrupole system. MRM was performed in positive electrospray ionization mode (ESI) using a BEH C18 UPLC column (2.1 × 50 mm, 1.8 mm; Waters, Milford, MA). The following MRM transition ions were monitored for fentanyl: quantifier: *m/z* 337.3 > 188.0, qualifier: *m/z* 337.3 > 105.0, and IS: *m/z* 342.0 > 188.1. Quantifier/qualifier ion ratio tolerance was set at 20% from the average of the calibrators. The analytical quality-control samples were prepared at 3 levels: 5, 30, and 75 ng/mL in K2-EDTA plasma.

Mobile phase A (MPA) was composed of 0.1% formic acid in 18.2 MW-cm H_2O, and mobile phase B (MPB) was composed of 0.1% formic acid in methanol. The flow rate was set at 0.800 mL/min across the entire method; the initial starting conditions were set at 95% MPA and 5% MPB. At 0.5 min, the composition of MBP increased linearly over 2.0 min to 61% MPB. At 2.5 min, MPB increased to 95% over 0.01 min and was held for 0.8 min before returning to 5% MPB over 0.01 min. The column was reequilibrated for the next 1.18 min at 95% MPA and 5% MPB for a total run time of 4.5 min.

Fentanyl was extracted from 150 µL of plasma spiked with 150 mL of IS. Proteins were precipitated using 450 mL of cold (−20 °C) CAN and vortexed for 30 s before centrifugation at 3,000 × *g* for 10 min at 4 °C. One hundred microliter of the supernatant was subsequently diluted with 900 µL of 18.2 MW-cm H_2O, and 10 mL was injected via the autosampler for LC-MRM analysis.

Determination of plasma buprenorphine concentrations by high-performance liquid chromatography tandem mass spectrometry. Calibrators were prepared from certified reference material: buprenorphine (Cerilliant catalog#: B-902). Internal standards (IS) was prepared from certified reference material: buprenorphine-d4 (Cerilliant catalog #: B-908). An external calibration curve using linear regression to plot the peak area ratio (Calibrator: IS) compared with concentration with 1/× weighting resulted in a linear curve, R2 30.99, over the full AMR. The AMR for the buprenorphine assay was 1.25 to 20 ng/mL.

HPLC MS/MS was performed in MRM mode using and Agilent 110 coupled to an API 4000 triple quadrupole mass spectrometer. MRM was performed in positive ESI mode using a MacMod Ace-5 C18 column (2.1 × 150 mm; MAC-MOD Analytical, Chadds Ford, PA). The following MRM transition ions were monitored: buprenorphine:

$$468.0 > 55.0\text{m} / \text{x}; \text{IS} : \text{m} / \text{z}472 > 55\text{m} / \text{z}.$$

Buprenorphine was extracted from 20 mL of plasma, and protein was precipitated with 60 mL of 100% acetonitrile containing buprenorphine-d4 IS. After centrifugation at 3,000 × *g* for 10 min, 10 mL of supernatant was injected into the LC-MS/MS system for analysis.

Fecal collection. Feces were collected from the cage liner at 1130 PST each day and weighed on a gram scale daily for the first 3 d before surgery to obtain a baseline average, on the day of surgery, and for the 4 d after surgery.

Pharmacokinetic analysis. Pharmacokinetic data were evaluated with noncompartmental analyses (Phoenix WinNonlin version 8.3; Certara, Princeton, NJ).

Statistical analysis. To determine the best model for predicting changes in fecal output, we first generated a baseline fecal output level for each rabbit in the study by calculating the mean fecal output from preoperative data. The fecal output data collected after surgery were then normalized to this preoperative baseline value that was determined for each rabbit by

subtracting the postoperative value from the preoperative baseline average. We then used the fBasics package in the R statistical programming language to determine that these differences from baseline data were normally distributed (D'Agostino's K^2: omnibus $P = 0.9512$, skewness $P = 0.8184$, kurtosis $P = 0.8276$) and appropriate for use in a linear model. Backward model selection was done using AIC on a fully crossed linear model containing the following factors: drug administered, postoperative day of recovery, and sex. AIC scores revealed that the removal of sex as a factor in the model did not decrease our ability to predict fecal production. Therefore, our proposed best model for our data is: fecal output approximately drug taken + postoperative day of recovery.

A similar approach was used for the facial grimace data. Average grimace scores from before and after surgery were compared for each rabbit. We then determined that the scores were normally distributed (D'Agostino's K^2: omnibus $P = 0.6836$, skewness $P = 0.5761$, kurtosis $P = 0.5031$) and appropriate for use in a linear model. Backward model selection using AIC was done using a fullycrossed linear model containing the following factors: drug administered, day of recovery, and sex. The AIC model selection again was used to determine the best model. The best predictor of changes in facial grimace was the postoperative day of recovery. Both sex and drug were removed from the model as they accounted for little of the variance.

For all models, α value was 0.05 and when appropriate, post hoc testing was done with Tukey's honestly significant difference.

Post hoc power analysis was used to determine the expected level of detection to determine whether the type II error rate would be artificially increased in a biologically meaningful way. Using the number of groups (4), the sample size in each group (5), the within-group variance (0.187), the α (0.05), and a standard power of 0.8, we determined that the level of between group variation that would have lead us to reject our null hypothesis was 0.174.

Results

Pharmacokinetics. Figures 1 and 2 show plasma concentrations of buprenorphine (administered as EXR) and fentanyl (administered as an FP) as a function of time after surgery. For EXR, an analgesic plasma concentration of buprenorphine (> 0.1 ng/mL) was measured at 4 h and maintained for 72 h for both sexes. Pharmacokinetic parameters of buprenorphine are shown in Table 2. For the transdermal FP, an analgesic plasma concentration of fentanyl (> 0.5 ng/mL) was surpassed at 8 h yet decreased below this threshold between the 24- and 48-h time points.

Fecal output. The fecal output of rabbits in the FP and EXR groups was evaluated for male and female rabbits for 3 d before surgery (baseline), on the day of surgery and for 4 d after surgery (Figure 3). Our statistical models did not find a sex-related difference in fecal output among rabbits that received the same drug. FP rabbits had an average of 30.8 g less fecal output than EXR rabbits. The model significantly outperformed the null model ($F_{9,90} = 2.471$, $P = 0.004429$) and accounted for 16% of the total variance in fecal output. Tukey honestly significant difference revealed that a significant increase in fecal output occurred between the day of surgery and day 4 after surgery, with an increase in fecal output of 55.2 g for both groups. On day 4 after surgery, EXR rabbits had a mean fecal output of 27.25 g, which is not a significant change from the baseline average ($t = 1.0763$; $df = 9$; $P = 0.3098$). In contrast, on day 4 after surgery for the FP group, the mean fecal output was −39.05 g, which is a significant decrease from baseline ($t = -2.65478$; $df = 9$; $P = 0.02657$). Therefore, EXR rabbits regained baseline output 1 day significantly faster than did FP rabbits. Fecal output for EXR rabbits returned to baseline at around 3 d after surgery, whereas FP rabbits did not return to baseline amounts until day 4 after surgery.

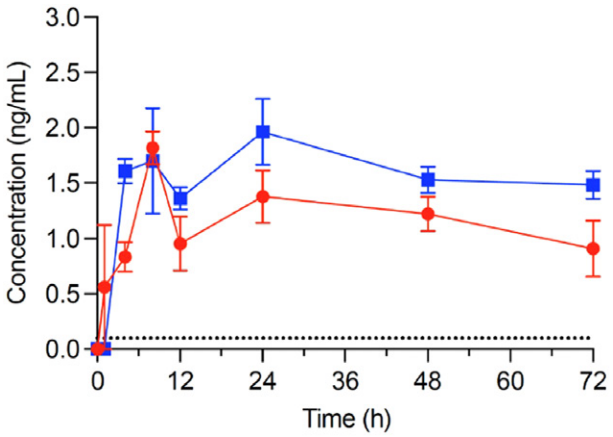

Figure 1. Median and standard error of plasma buprenorphine concentrations over 72 h after subcutaneous injection of 0.15 mg/kg EXR. The dotted line indicates the analgesic target concentration (0.1 ng/mL). Red circles indicate male rabbits; blue squares indicate female rabbits.

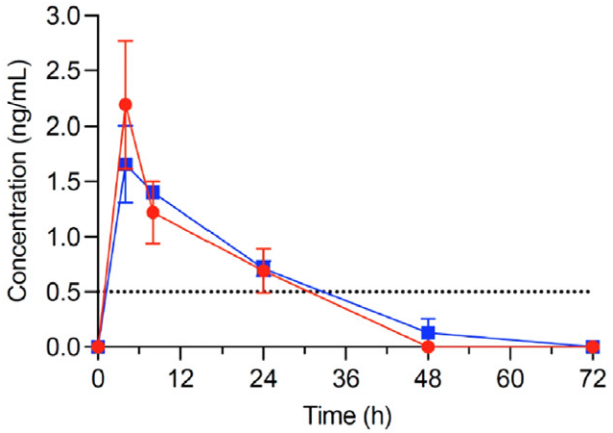

Figure 2. Median (standard error) plasma fentanyl concentrations (ng/mL) over 72 h after placement of a transdermal 12 µg fentanyl patch. The dotted line indicates the analgesic target concentration (0.5 ng/mL). Red circles indicate male rabbits; blue squares indicate female rabbits.

Table 2. Pharmacokinetic parameters for buprenorphine after subcutaneous administration of 0.15 mg/kg of EXR

	Female		Male	
Variable	Geometric mean	%CV	Geometric mean	%CV
AUC last (h*ng/mL)	111.89	18.44	77.22	37.26
C_{last} (ng/mL)	1.46	18.81	1.16	24.01
C_{max} (ng/mL)	2.18	19.85	2.04	23.45
T_{max} (h)	15.47	66.05	7.55	235.59

%CV, coefficient of variability of pharmacokinetic parameters of EXR; AUC, area under the curve to the time of last measured concentration; C_{last}, last measured concentration; C_{max}, maximum measured concentration; T_{max}, time of maximum concentration.

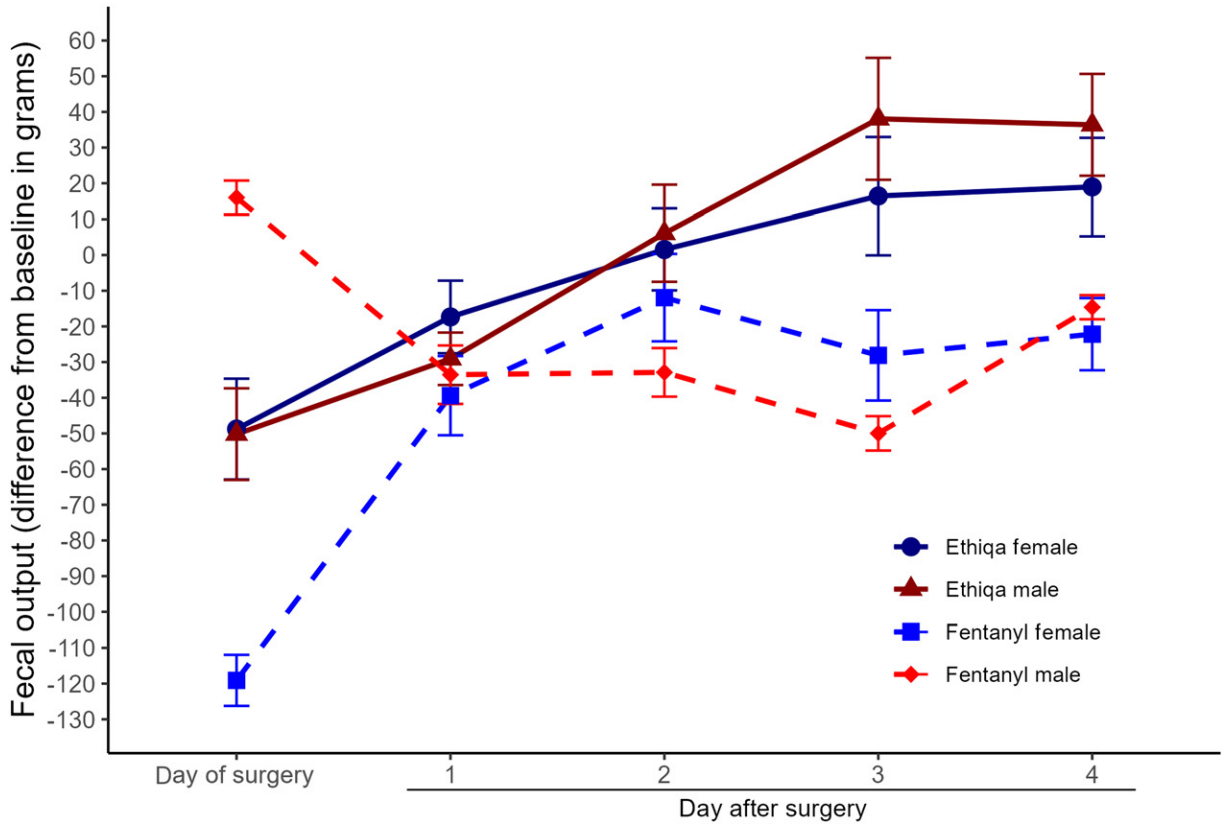

Figure 3. Fecal output (difference relative to baseline for the day of surgery day and the first 4 d after surgery in rabbits that received either EXR or a fentanyl patch. Each rabbit served as its own baseline (baseline value minus test value).

Behavioral observation. Rabbits were monitored with a camera for 2 d before surgery, on the day of surgery, and for 3 d after surgery to assign grimace scores. Each rabbit's postoperative scores were compared with their own individual baseline scores to determine whether pain attributable to surgery was present. Scores were assessed based on both treatment and sex (Figure 4). Our statistical model did not detect a difference in grimace score based on either sex or drug treatment. The only significant predictor of grimace score was day of recovery, with rabbits showing significant postsurgical improvement only on days 1 and 3 after surgery ($F_{3,73}$ = 3.883; P = 0.01239).

Discussion

This study demonstrates that a single subcutaneous injection of EXR provides buprenorphine plasma concentrations above the analgesic target concentration (0.1 ng/mL) for 72 h in rabbits. For the transdermal FP, plasma concentrations fell below the analgesic target concentration of fentanyl (> 0.5 ng/mL) between 24 and 48 h after surgery, indicating a shorter duration of analgesia. Rabbits did show an initial increase in grimace score from baseline on the day of surgery, but, on recovery days, there was no significant change in grimace score from baseline showing that rabbits were not painful postoperatively. Grimace scores showed no significant differences among treatment groups, indicating a lack of behavioral evidence for superior pain control by one of the 2 treatments. The fecal output data showed no significant difference in fecal output between treatment groups. The fecal output of rabbits that received EXR may have returned to baseline sooner than those that received the FP. Data from this limited number of rabbits indicate that EXR dosed at 0.15 mg/kg SC is suitable for use in rabbits and has minimal side effects.

Although buprenorphine can reduce gastric motility and appetite in rabbits, this effect can be subtle.[4,6] A study that compared buprenorphine and meloxicam in Dutch-Belted rabbits reported that both drugs had similar effects on gastric motility.[4] Another study showed that a single high dose of buprenorphine had no adverse effect on gastrointestinal motility in healthy rabbits.[6] Our findings align with these studies, as they indicate that EXR and FP have comparable effects on appetite and gastric motility. Previous studies have suggested that postoperative reductions in fecal output may be linked to reduced food intake rather than pain or handling stress.[6,10,15] However, coprophagia could also influence the fecal output data. The main factors driving the reduction in fecal output after surgery in this study are likely to be a reduced appetite and a subtle decrease in gastric motility caused by the opioid medications. Future studies could determine which factor—stress, opioids, or food consumption—has the largest influence on fecal output. Despite these considerations, both drugs had limited side effects and are appropriate for use in rabbits. None of the rabbits in this study developed gross injection site granulomas; however, histopathology was not performed. All 20 rabbits reached the study endpoint, indicating the overall safety of the drugs.

Our data did not reveal any significant difference between grimace scores between EXR and FP rabbits. Based on this, we found no indication that one analgesic was significantly superior to the other in controlling postoperative pain in rabbits. However, our study has several limitations. First, we tested only 5 rabbits per group, and results must be interpreted with this in mind. A post hoc power analysis revealed that our study was underpowered to detect small changes (any changes smaller than ±0.174) on the 2-point grimace scale. Although a change in grimace score of 0.174 might be statistically significant, it is

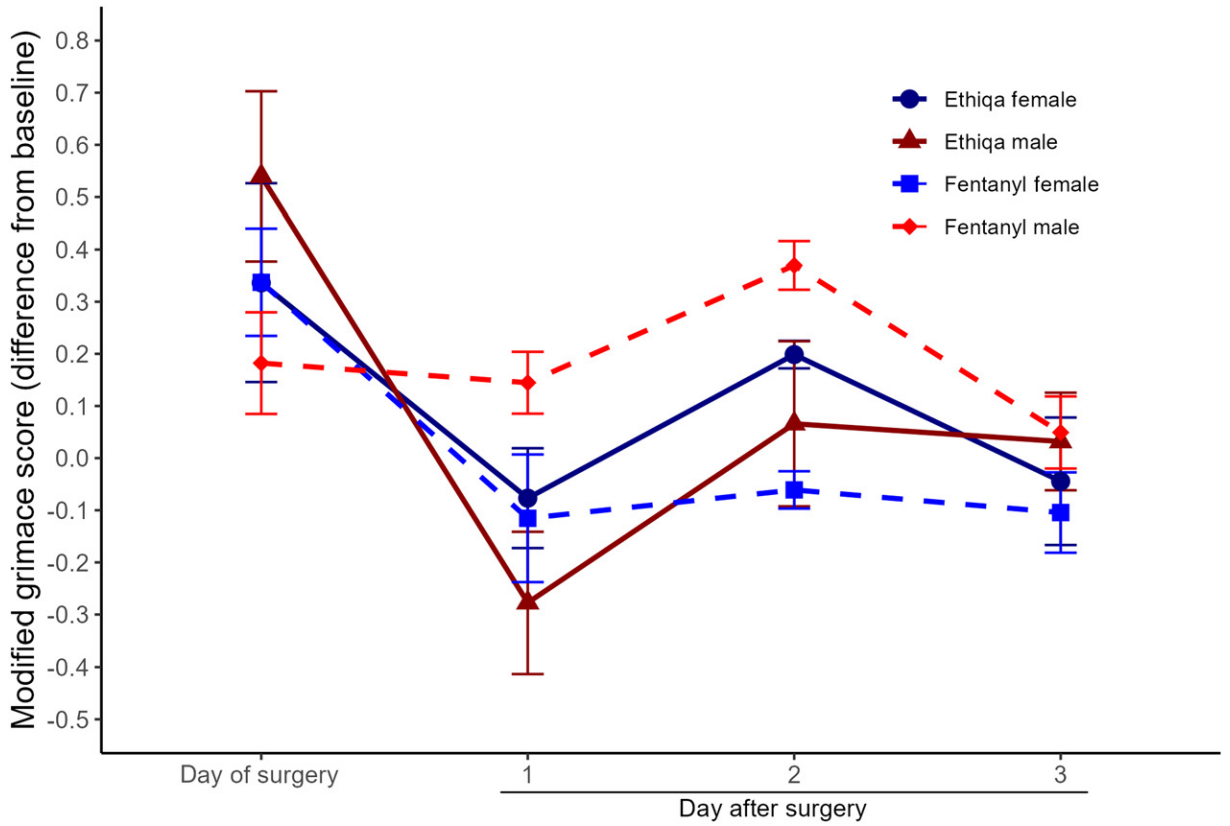

Figure 4. Grimace score (difference relative to baseline for the day of surgery day and the first 3 d after surgery in rabbits that received either EXR or a fentanyl patch. Each rabbit served as its own baseline (baseline value minus test value).

probably not clinically significant enough to indicate the need for additional analgesia. The detection of smaller significant smaller differences in grimace scores between groups would require additional research with a larger sample size. Furthermore, the 15-min recording periods did not continuously capture the rabbits' faces, as they sometimes turned away from the camera. Moreover, some rabbits were seen chewing the cameras, which obstructed the view of their facial expressions. Another potential concern was that the blinking light on the camera may have interfered with the rabbits' natural behaviors. One study showed that buprenorphine-treated rabbits may display more abnormal behaviors (for example, crouching and sitting) as compared with rabbits that received lidocaine analgesia.[22] Follow-up experiments could evaluate grimace scores with nonblinking cameras, compare behaviors of normal (pain-free) and postoperative rabbits given the same analgesia, and investigate alternative noninvasive techniques that do not interfere with the natural behaviors of rabbits.

Rabbits that received EXR at 0.15 mg/kg SC maintained analgesic levels of buprenorphine for 72 h regardless of sex. In contrast, a study in rats that reported sex differences in buprenorphine metabolism that were significant enough to suggest the use of different dosing.[13] Although male and female rabbits have been reported to differ with regard to opioid receptors, metabolism, and sex hormone levels, these differences may not be large enough to require sex-based variations in drug dosing.[19] This conclusion is consistent with another study on sex differences in rabbit pharmacokinetics that concludes that sex-based drug dosing of rabbits may be complicated by the complexity of drug metabolism, drug interactions, and genetic variation.[19] Body size may be the main factor in EXR dosing for rabbits regardless of sex.

In the present study, we observed that FP could detach from the rabbits' skin. Consequently, a significant proportion of our experimental subjects required the application of additional skin glue to maintain patch adherence. These findings align with a previous report of patch detachment, which resulted in the death of 2 rabbits due to the ingestion of the detached patch.[18] The inadequate adhesion of FP could certainly compromise the accuracy and consistency of drug delivery because of variability in absorption. Other factors that could influence drug absorption from an FP include the amount of subcutaneous fat under the patch, the integrity of the skin, body temperature, and arrangement of the hair follicles.[9,17] These factors could contribute to the variability in plasma levels of fentanyl observed in rabbits in this study.

The cost of using EXR use far exceeds that of using the FP. A 3-mL vial of EXR costs $415 and can be used to treat approximately 4 rabbits. Twenty-four FPs can be purchased for $23 and can be used for 24 rabbits. The cost per rabbit for EXR is approximately $104, whereas the cost per rabbit for an FP is approximately $1 per rabbit. Our EXR pharmacokinetic data show that administration of EXR at 0.15 mg/kg SC results in plasma concentrations of buprenorphine that exceed the analgesic threshold of 0.1 ng/mL for up to 3 d. Future studies could evaluate the pharmacokinetics of a lower dose of EXR for rabbits, which could reduce the cost of this formulation for providing adequate analgesia.

Although our study had a sample size of only 5 rabbits per group, our data suggest that EXR may have several advantages over the FP. One advantage is the ability to inject the analgesic preoperatively. In our study, the injectable formulation reached adequate analgesic plasma levels more consistently than did the FP. Although the FP patch may provide a more cost-effective

option, the potential benefits and drawbacks of each analgesic regimen must be considered, including issues of patch detachment and animal welfare, to support fully-informed decisions regarding analgesic strategies in rabbits.

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Conflict of Interest

The author(s) have no conflict(s) of interest to declare.

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