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Pharmacokinetic and Histopathologic Study of an Extended-Release, Injectable Formulation of Buprenorphine in Sprague–Dawley Rats

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A novel buprenorphine (BUP) extended-release formulation (BUP-XR) produced as a lipid-encapsulated, low viscosity BUP suspension for SC injection to control pain was evaluated for pharmacokinetics and safety in Sprague–Dawley rats given either 0.65 mg/kg (low dose) or 1.30 mg/kg (high dose). The 2 dosage groups each contained 6 male and 6 female rats to determine whether BUP-XR behaved differently in male or female animals. Blood samples were obtained from each animal before BUP-XR administration and at 6, 24, 48, 72, 96, and 168 h after administration. For necropsy and injection-site histopathology evaluation, 3 animals of each sex from each test group were euthanized on day 8, with the remaining animals euthanized on day 15. Mean plasma BUP concentration peaked from 6 to 24 h in all test groups, then declined in a linear fashion. Quantifiable plasma BUP was measured in all male rats at all time points except for one low dose group sample taken at 168 h. Female rats had quantifiable plasma BUP at all time points except for 1 low dose group sample at 72 and 96 h, and 2 low dose group samples at 168 h. The low dose groups, whether male or female, had lower mean plasma BUP levels at all time points as compared with their high dose counterparts, and female rats had lower mean plasma BUP levels than male rats at all time points. Results indicate that a single BUP-XR dose at either dose concentration can reliably provide plasma levels of BUP reported in the literature to be therapeutically relevant for up to 72 h, although lower plasma BUP levels can be anticipated in female rats compared with male counterparts. Mild to moderate injection-site granulomatous inflammation was observed in 6 of 12 rats in the low dose group and 7 of 12 in the high dose group. This reaction is characteristic of lipid material designed to persist in situ.

Abbreviations: BUP, buprenorphine HCl; BUP-SR, sustained-release formulation of buprenorphine; BUP-XR, extended-release formulation of buprenorphine; PK, pharmacokinetic; SR, sustained-release

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Analgesia is an essential component in the management of laboratory rats and mice used in research studies that involve pain. The 2 most commonly used classes of systemic analgesics are NSAIDs and opioids.[24,25] Because of the therapeutic variability and side effects of NSAIDs, they are increasingly recommended for use with opioids in multimodal protocols to potentiate efficacy and minimize the dosage of the individual drugs used.[24,25] Therefore, opioids are widely used in laboratory animals because of their potent analgesia, efficacy in a wide range of moderate to severe pain models, parenteral dosage form, high therapeutic index, and well defined but minimal side effects when used appropriately, including minor effects on immune function when used acutely.[3,6,7,11,16] Surveys confirm that buprenorphine (BUP) is the most widely used opioid in rodents, and based on decades of safe and effective use is the analgesic of choice in many if not most research laboratories.[3,8,11,21] The standard dose for BUP in laboratory rodents is 0.05 mg/kg administered subcutaneously (SC) at least every 12 h.[3,5] The 6 to 12 h duration of action of BUP is an important limitation because maintenance of analgesia requires repeated handling and restraint of animals for up to 3 daily doses, with associated stress to the animal and demands on personnel.[13] Some studies report that BUP given at the label dosage routinely does not achieve a therapeutic plasma level beyond 4 to 6 h in rodents.[7,17]

A commercial, compounded sustained release (SR) parenteral formulation of BUP (BUP-SR) for use in rodents, first described in 2011, overcame the inconvenience and limited duration of effect of formulations requiring BID or TID administration.[3,8,16,24] Depending on the model, BUP-SR given at a dosage of 0.65 mg/kg maintained therapeutic BUP plasma concentrations of 1 ng/mL for 48 to 72 h in rats.[7,8] Thus, BUP-SR formulation achieved the clinical goals of dosing at multiday intervals, less need for animal handling, restraint and stress, decreased personnel time; and avoidance of lapses in therapeutic BUP plasma concentrations.

Preliminary findings in our lab suggested that BUP levels might be lower in female Sprague–Dawley rats as compared with male rats given the same dose. We therefore sought to test this relationship and to determine whether a higher dose would achieve more desirable levels in female Sprague–Dawley rats. The current data establish the pharmacokinetic (PK) profile in rats of a novel BUP extended-release formulation (BUP-XR) produced as a lipid-encapsulated (primarily cholesterol), low viscosity BUP suspension administered by SC injection. The gradual in situ diffusion of BUP afforded by this formulation allows relatively large doses to be safely administered and

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provides a prolonged therapeutic effect. We measured plasma BUP concentrations for 7 d after administration of one of 2 BUP-XR doses: 0.65 mg/kg or 1.30 mg/kg. The data showed that BUP maintained a long-term effective concentration in vivo in the outbred Sprague–Dawley rat, a stock used extensively in medical research. A previous study had used the inbred Fischer 344/NTac rat.[10] Our study also evaluated whether BUP-XR PK differed in male and female rats. An adjunct to the PK study was the necropsy of the test animals and histopathologic evaluation of injection site.

Materials and Methods

Test animals and husbandry. A total of 24 (12 male, 12 female) albino Sprague–Dawley rats (Charles River, Raleigh, NC) from a colony certified to be free of common specific pathogens were used as test animals. At the onset of the study, the rats were approximately 10 wk of age and weighed from 243 to 294 g for males (mean ± SD, 267 g ± 17) and 192 to 242 g for females (mean, 216 g ± 16). Rats were acclimated to laboratory conditions for 12 d prior to administration of the BUP-XR agent.

Test animals were maintained in accordance with a protocol developed by the Institutional Animal Care and Use Committee (IACUC) of the research organization conducting the study (SoBran BioScience, Browns Summit, NC). The protocol (515-001) was consistent with the provisions of the PHS Policy on Humane Care and Use of Laboratory Animals[18] and the U.S. Interagency Research Animal Committee Principles for the Utilization and Care of Research Animals and was approved by SoBran's IACUC. Diet, environmental conditions, and enrichment provisions were in accordance with National Research Council guidelines.[12,21] Rats were housed 3 per cage by sex for acclimation and then in individual cages for the duration of the study to avoid the possibility of the rats injuring each other and thereby interfering with observation of the injection sites.

Rats were housed in ventilated microisolation cages (Innovive, San Diego, CA) with Alpha-Dri bedding (Shepherd Specialty Papers nonwood chip, Watertown, TN) on ventilated racks (Innovive, San Diego, CA). Bedding and cages were changed at least once weekly. Feeders and cage lids were changed at least once every 2 wk. Temperature and humidity were monitored daily and recorded by the facility staff. Rats were housed in an environmentally controlled room that maintained temperatures in the range of 20.0 to 26.1 °C (68 to 79 °F) and relative humidity between 30% to 70%. Fluorescent lighting was on an automatic schedule to provide a 12:12 h light:dark cycle. All rats received ad libitum access to Envigo Harlan Teklad Rodent Diet 2016 (Indianapolis, IN) and to tap water via water bottles. Nylabones were provided as an appropriate and approved enrichment device according to facility SOPs.

Test article. The BUP-XR agent was a proprietary formulation of USP-grade buprenorphine HCl suspended in a medium-chain triglyceride oil (Ethiqa XR, Fidelis Pharmaceuticals, North Brunswick, NJ). The formulation was packaged as a sterile, ready-to-use suspension at a BUP concentration of 1.3 mg/mL. The same BUP-XR production lot was used in all test animals. The test agent was stored at room temperature and briefly mixed by repeated vial inversion prior to each use. The BUP-XR agent was administered subcutaneously in the dorsal space between the scapulae using a 22-gauge needle.

Study design. Rats in each sex grouping were randomized by body weight for assignment to BUP-XR low dose (the label dose) or high dose (2× the label dose) test groups (n = 12 per group, 6 male and 6 female). Rats were individually identified with uniquely numbered stainless steel ear tags at the time of randomization. A computer-generated randomization program was used to allocate test animals to their respective groups. The intragroup body weights varied by < 20% for each sex grouping. Body weights obtained on day 1 prior to BUP-XR administration were used to calculate the individual dose volume for each rat. On day 1, BUP-XR was given at a dose of 0.65 mg/kg body weight to the low dose group and at a dose of 1.30 mg/kg to the high dose group.

A 0.3 mL blood sample was obtained from the jugular vein from each rat before BUP-XR administration on day 1 and at 6, 24, 48, 72, 96, and 168 h after administration. Blood was collected using a 3 mL syringe with a 25G needle and a rat restraint board that was developed inhouse. Rats were placed on the restraining board and a needle was inserted into the jugular. The blood was transferred from the syringe to a 0.5 mL K3 EDTA blood tube, the blood tube was inverted several times and was then placed on wet ice until it was processed to plasma. Blood samples were centrifuged to obtain plasma, which was stored in individual cryovials for frozen storage (-80 °C) until shipped for laboratory analysis. In case of samples with a volume < 100 µL (5 in all), control rat plasma was added, and the plasma sample concentration was corrected for dilution. If a plasma BUP concentration was below the quantitation limit (BQL) of < 0.2 ng/mL but above the lower limit of detection, a value of 0.1 ng/mL (one-half the BQL) was used in the calculation.

Sample analyses were performed by HPLC-MS/MS using Analyst 1.6.2 software (AB Sciex Foster City, CA) in the positive ion mode using mixed reaction monitoring (buprenorphine: m/z 468.4 to 396.1, and terfenadine 472.4 to 436.2). Analyte quantifications were obtained using matrix-matched calibration standards with internal standardization along with QC samples at 3 levels per calibration in triplicate. The PK values were calculated using PK Solver 2.0 and included peak plasma concentration of the BUP analyte, time to peak plasma concentration, plasma half-life, area under the plasma concentration-time curve, clearance, apparent volume of distribution, and elimination rate constant (C_{max}, T_{max}, $T_{1/2}$, $AUC_{0\text{-}t}$, CL, Vd, and Ke, respectively). A linear mixed effects ANOVA model was then used to compare the difference in body weight between day 1 and day 8, day 1 and day 15 and AUC (t = 0 to 168 h) for the effects of dose group, sex, and the interaction of dose group by sex for the study samples. All statistical analyses were completed using SAS Version 9.4 (SAS Institute, Cary, NC).

For purposes of tissue collection, half of the rats (3 animals of each sex from each test group) were euthanized on day 8 by CO_2 inhalation and thoracotomy followed by exsanguination. The same euthanasia procedure was performed on day 15 for the remainder of the test rats.

Clinical observation, necropsy, and injection-site histopathology. During the acclimation period, rats were handled for 3 to 5 min at least once daily and were clinically evaluated for health status at each handling. Beginning on day 1 and for the remainder of the study, hands-on examinations were performed twice daily for each rat to detect injection-site reactions, overall health and appearance, signs of BUP-XR toxicity, and any other morbidities. Specific attention was given to signs suggestive of BUP-associated adverse reactions, including gastrointestinal or urologic abnormalities, nausea, food intake aberrations, distended abdomen, excessive grooming or chewing on forelimbs,

and signs of pica. A final hands-on clinical evaluation was performed for each rat prior to euthanasia.

After euthanasia, necropsies were performed on all test rats. External body surfaces, all orifices, the cranial cavity, thoracic cavity, and peritoneal cavity were examined. Tissues from gross lesions were collected, individually labeled, and preserved in buffered formalin for histopathologic evaluation. Injection-site tissue was collected from all rats, individually labeled, and preserved in formalin for histopathologic evaluation. Preserved injection-site tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and microscopically evaluated for granulomas and other cellular lesions.

Results

Clinical outcomes. After BUP-XR administration, all test rats survived until euthanized by CO_2 at day 8 or after completion of the study at day 15. Mean body weights by group reflect normal weight gain over the 1 to 8 and 1 to 15 d periods (Table 1). Three individual rats, 2 high dose group males and one low dose group female, lost weight from days 1 to 8, with the loss ranging from 0.6 to 5.7 g. A high dose group male rat that lost 5.7 g at day 8 had a 43.4 g weight gain by day 15. A low dose female that lost 1.0 g on day 8 had a 12.8 g weight gain by day 15. By day 15, none of the remaining test animals had a weight loss.

In the low dose group, male rats showed no clinical signs after BUP-XR administration. Several clinical signs occurred after administration in rats from other test groups, as summarized in Table 2. Two low dose females showed forelimb lameness on days 4 and 5. Local infection and exudate at the ear-tag site were observed in 2 low dose females on day 6. Three low dose females had cloudy urine and urinary sediment on day 8, and one low dose female had vaginal discharge on day 11.

In the high dose group, 2 male rats developed forelimb lameness beginning on day 1 and lasting 4 to 8 d. Five males had cloudy urine and urinary sediment on day 8. One male rat had an abdominal wound on day 5 that began to heal by day 7. The same male rat had skin sloughing at the BUP-XR injection site. Individual high dose females had foreleg lameness from day 4 to 8, skin irritation or sores on paws from day 5 to 6, local infection at the ear-tag site on day 6, forelimb scabs on day 8, and vaginal discharge on day 11. Four high dose females had cloudy urine, opaque urine, or urinary sediment on day 8 and day 15, and 2 high dose females had forelimb alopecia on day 7.

Pharmacokinetic analysis. Samples obtained on day 1 prior to BUP-XR administration and at 6, 24, 48, 72, 96, and 168 h after administration were analyzed for each of the 24 test rats. A total of 163 plasma samples were collected, with missing samples for 5 female rats (2 in the low dose group and 3 in the high dose group) at 168 h. Mean PK values by test group and sex are shown in Table 3. PK parameters were analyzed using a mixed model with the effects of dose, sex and dose by sex interaction. All statistical analyses were completed using SAS Version 9.4 (SAS Institute, Cary, NC,). Some PK values differed between doses and sexes. Male and female rats in the high dose groups had a significantly greater mean C_{max} (P = 0.014) and mean $AUC_{0\text{-}t}$ (P = 0.001) values than did the low dose groups. Mean C_{max} was 61% to 70% greater in the high dose groups and mean $AUC_{0\text{-}}t$ in the high dose groups was approximately double that of the low dose groups. Exposure to a higher BUP-XR dose translated into 53% longer mean $t_{1/2}$ times for the high dose groups as compared with low dose groups. The mean volume of distribution was 20% to 27% greater and the mean elimination rate constant was approximately 6-fold greater in the high dose groups as compared with the low dose groups. The mean clearance rate was 20% to 28% slower in the high dose groups as compared with the low dose groups. In male rats, mean T_{max} was equivalent between the dosage groups (24 h). In female rats, the concentrations at 6 and 24 h were not statistically different.

Table 1. Mean body weights by test group

	Study interval and mean body weight (g) ± SD.			Mean body weight change (g) ± SD.	
Test group	Day 1	Day 8	Day 15	Days 1–8	Days 1–15
Low male	269 ± 17 (n = 6)	283 ± 15 (n = 6)	326 ± 23 (n = 3)	14 ± 6 (n = 6)	65 ± 11 (n = 3)
High male	265 ± 17 (n = 6)	275. ± 17 (n = 6)	333 ± 4 (n = 3)	10 ± 13 (n = 6)	60 ± 15 (n = 3)
Low female	221 ± 12 (n = 6)	229 ± 16 (n = 6)	244 ± 5 (n = 3)	8 ± 10 (n = 6)	22 ± 9 (n = 3)
High female	210 ± 19 (n = 6)	223 ± 22 (n = 6)	223 ± 2 (n = 3)	13 ± 3 (n = 6)	24 ± 13 (n = 3)

BUP-XR dosages = 0.65 and 1.3 mg/kg body weight in low dose group and high dose group, respectively.

Table 2. Clinical sequelae following administration of extended-release buprenorphine (BUP-XR)

	Test group and incidence of clinical signs			
Clinical sign	Low dose males	High dose males	Low dose females	High dose females
Forelimb lameness	No clinical signs	2 of 6	2 of 6	1 of 6
Ear-tag inflammation			2 of 6	1 of 6
Urinary sedimentation		5 of 6	3 of 6	4 of 6
Vaginal discharge			1 of 6	1 of 6
Abdominal mass and wound		1 of 6		
Injection-site dermatoses		1 of 6		
Paw dermatoses				1 of 6
Forelimb scab or alopecia				3 of 6

BUP-XR dosages = 0.65 and 1.3 mg/kg body weight in low dose group and high dose group, respectively.

Table 3. Summary of mean pharmacokinetic (PK) values following BUP-XR subcutaneous injection

	Test group and PK values			
PK parameter (unit)	Low dose males	High dose males	Low dose females	High dose females
$AUC_{0\text{-}t}$ (ng/mL*h)	125.6 (±56.8)	249.7 (±77.8)	85.9 (±33.6)	174.9 (±58.3)
$T_{1/2}$ (h)	51.0 (±37.7)	78.1 (±72.7)	55.4 (±19.1)	85.1 (±45.5)
CL (L/h)	1.24 (±0.40)	1.03 (±.32)	1.46 (±1.2)	1.14 (±0.27)
Vd (L)	91.3 (±66.9)	115.6 (±89.2)	116.4 (±71.9)	140.3 (58.1)
Ke (h^{-1})	0.014 (±0.008)	0.089 (±0.004)	0.013 (±0.006)	0.081 (0.03)
T_{max} (h)	24 (±16.7)	24 (±13.4)	6 (±3.9)	24 (±9)
C_{max} (ng/mL)	1.58 (±1.0)	2.69 (±1.1)	1.12 (±0.34)	1.81 (±0.44)

BUP-XR dosages = 0.65 and 1.3 mg/kg body weight in low dose group and high dose group, respectively. Values in parenthesis represent the standard deviation.

Table 4. Mean plasma BUP concentrations at BUP-XR after administration

	Test interval and mean BUP concentration in ng/mL (± SD)					
Test group	6 h	24 h	48 h	72 h	96 h	168 h
Low male (n = 6)	1.25 (0.62)	1.58 (1.16)	1.06 (0.58)	0.79 (0.36)	0.53 (0.23)	0.21 (0.06)
High male (n = 6)	1.90 (0.45)	2.69 (1.22)	2.12 (0.99)	1.56 (0.61)	1.24 (0.24)	0.76 (0.26)
Low female (n = 6)	1.12 (0.50)	0.98 (0.45)	0.73 (0.46)	0.51 (0.25)	0.34 (0.17)	0.15 (0.08)
High female (n = 6)	1.73 (0.58)	1.81 (0.59)	1.15 (0.40)	1.17 (0.56)	0.87 (0.55)	0.52 (0.13)

BUP-XR dosages = 0.65 and 1.3 mg/kg body weight in low dose group and high dose group, respectively.

Some mean PK values also differed between sexes (Table 3). Female rats in both dosage groups had 46% to 75% lower mean $AUC_{0\text{-}t}$ and 41% to 49% lower mean C_{max} values as compared with male rats. Female rats had a mean clearance rate that was 11% to 18% slower and a mean volume of distribution that was 21% to 28% greater than that of male rats. Mean BUP half-life and the mean elimination rate constant were roughly equivalent between sexes.

BUP was not detected in any of the pretreatment plasma samples. Mean plasma BUP concentration peaked at 6 to 24 h in all test groups, then fell linearly for the remainder of the study (Table 4). Plasma BUP could be quantified in all samples from male rats at all time points except for one low dose sample that was BQL at 168 h. Plasma BUP was less consistently detected in female rats given the low dose. In that group, BUP was BQL in 1 of 6 individuals at 72 h and 96 h, and 2 of 6 rats at 168 h. All low dose females had quantifiable plasma BUP at 6, 24, and 48 h. All samples from high dose females had quantifiable BUP at all time points.

The low dose groups, whether male or female, had lower mean plasma BUP levels at all time points as compared with their high dose counterparts, and female rats had lower mean plasma BUP levels than male rats at all time points (Figures 1 and 2) . The difference in mean BUP levels between high and low dose groups within sexes varied from 52% (1.25 compared with 1.90 ng/mL) between male dosage groups at 6 h to 3.56-fold (0.15 compared with 0.52 ng/mL) between female dose groups at 168 h (Table 4). The difference between males and females within dosage groups varied from 10% (1.90 compared with 1.73 ng/mL) in high dose groups at 6 h to 84% (2.12 compared with 1.15 ng/mL) in low dose groups at 48 h (Table 4).

A test for proportionality for the AUC adjusted by dose (AUC/dose) was performed using an ANOVA model. A statistically significant difference was not seen in the AUC/dose ratio between the 2 dose groups (P = 0.762), indicating dose linearity. The mean ratio between the AUC of the 2 dose groups was 1.96 (95% CI of 1.18 to 3.26), indicating that the AUC (0 to t = 168) for the high dose was twice as high as for the low dose. We were also able to show a statistically significant difference for the AUC/dose ratio between males and females (P = 0.019). No difference was detected for the dose by sex interaction (P = 0.841). Male rats had a higher average AUC/dose than did female rats (P = 0.019).

Macroscopic observations and injection-site histopathology. Macroscopic lesions in uninjected locations were unremarkable at necropsy on day 8 or day 15 and were considered to be unrelated to BUP-XR treatment. A urinary bladder calculus was found in a high dose male and was considered to be a spontaneous incidental lesion. Sternal discoloration in a low dose female and minor tissue trauma in the ventral thoracic and axillary muscle of a high dose female were likely due to procedural or handling trauma.

Mild to moderate subcutaneous (SC) granulomatous inflammation was macroscopically observed at the injection site for 6 of 12 low dose rats and 7 of 12 high dose rats. These abnormalities were noted on day 8 in 1 male and 2 females in the low dose group and in 3 males and 2 females in the high dose group. On day 15, injection-site lesions were noted in 1 male and 2 females in the low dose group and in 1 male and 1 female in the high dose group.

Microscopically, injection-site reactive changes after administration of the 0.65 mg/kg BUP-XR dose typically consisted of minimal perivascular infiltration of lymphocytes and plasma cells (Figure 3). Inflammatory regions consisted of nodular or multinodular aggregates of activated macrophages, smaller numbers of eosinophils, and variable central cavitation. As a component of the inflammatory process, scant lymphocytic infiltration was noted at the injection sites of 1 high dose group female on day 8 and 3 low dose group rats on day 15. After administration of the 1.30 mg/kg BUP-XR dose, injection site inflammation involved formation of granulomas associated with lipid material (Figure 4).

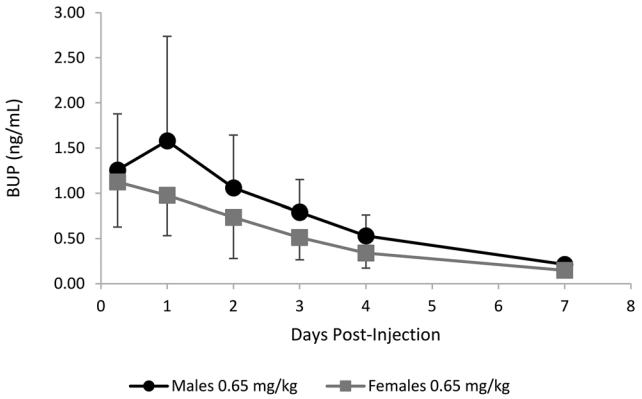

Figure 1. The mean plasma BUP concentrations (ng/mL) over a 7 d (168 h) period after injection in male and female rats (n = 6 per group) at BUP-XR dosage of 0.65 mg/kg (low dose).

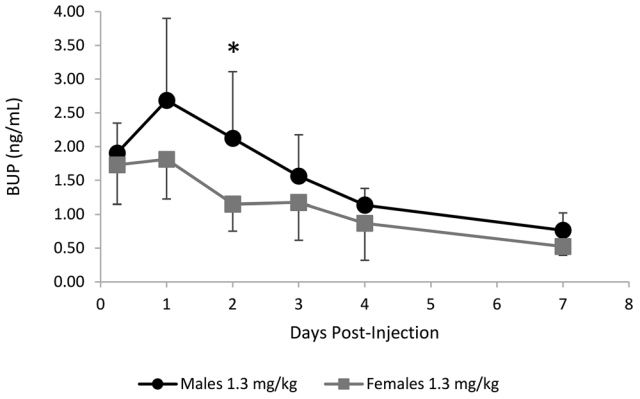

Figure 2. The mean plasma BUP concentrations (ng/mL) over a 7 d (168 h) period after injection in male and female rats (n = 6 per group) at BUP-XR dosage of 1.3 mg/kg (high dose). (* = point difference significant at $P < 0.05$).

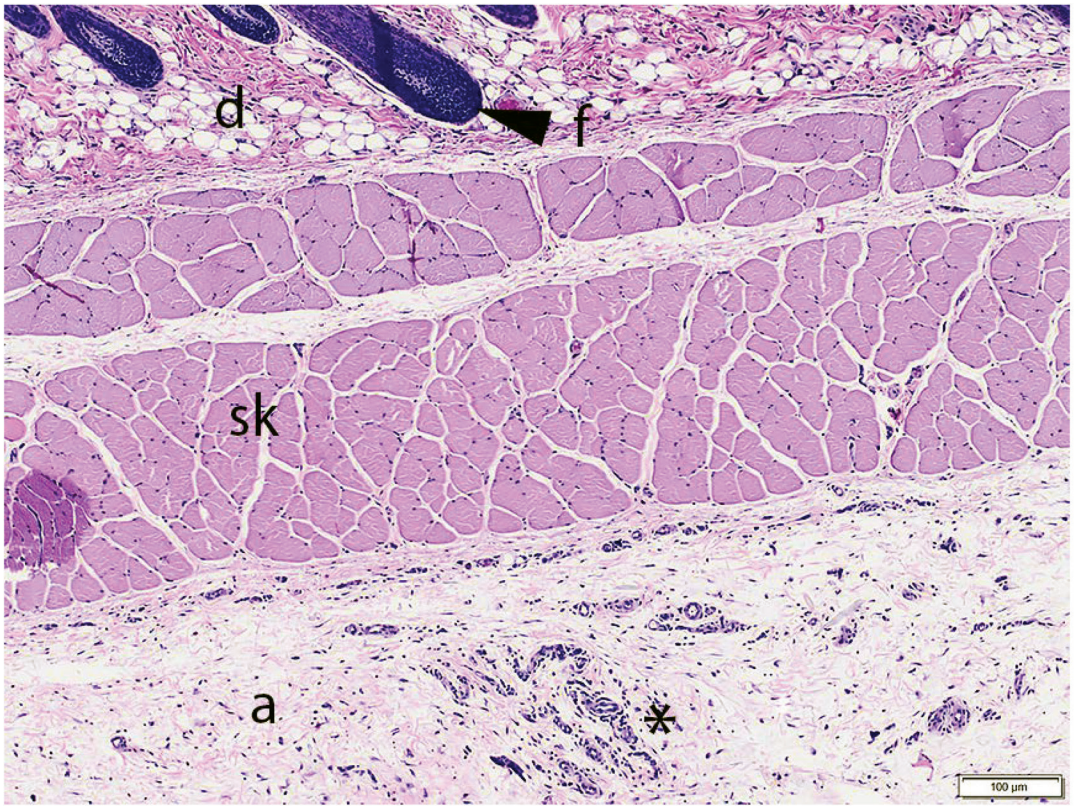

Figure 3. Skin from a Sprague–Dawley rat is shown at 40× total magnification, H and E stain, on day 15 following SC administration of 0.65 mg/kg (low dose) extended-release buprenorphine (BUP-XR). Scant perivascular infiltrate (asterisk) of lymphocytes and plasma cells is visible in the adventitia (a) at the injection site. d = dermis, f = hair follicle, sk = skeletal muscle (panniculus carnosus).

Discussion

The central finding of this study was the sustained therapeutic plasma BUP concentrations after parenteral administration of BUP-XR at either low or high doses. Within 6 h after SC administration (the earliest time point measured), all groups had mean plasma concentrations > 1 ng/mL, which were maintained in the high dose male rats Table 3 for at least 96 h. Detectable levels of BUP were present in 11 of 12 male rats until the 168 h time point and in 10 of 12 female rats at 96 h. Our long-term residual BUP plasma concentrations were similar to those of a prior study showing detectable but subtherapeutic plasma BUP levels for up to 9 d in rats after administration of a BUP-SR formulation given SC at 1.2 mg/kg.[19] Although earlier studies established the safety and efficacy of BUP-XR prototype formulations in target species,[4,9,10,13,22,23,26] ours is the first report of a sex difference in its PK parameters in laboratory animals.

Although plasma BUP concentrations of 1 ng/mL are generally considered to provide therapeutic analgesia in rodents,[7,8,24] the relationship between plasma BUP concentration and analgesic effect has not been definitely established and varies among species.[8,26,28] For example, in humans the target plasma BUP concentration is 0.5-0.7 ng/mL but the minimal effective concentration is considered to be 0.1 ng/mL.[8] Other investigators have found BUP plasma levels of 0.5 ng/mL to be clinically significant in various species, including humans and laboratory mice and rats.[9,20,26] Clinically relevant analgesia is likely achievable within

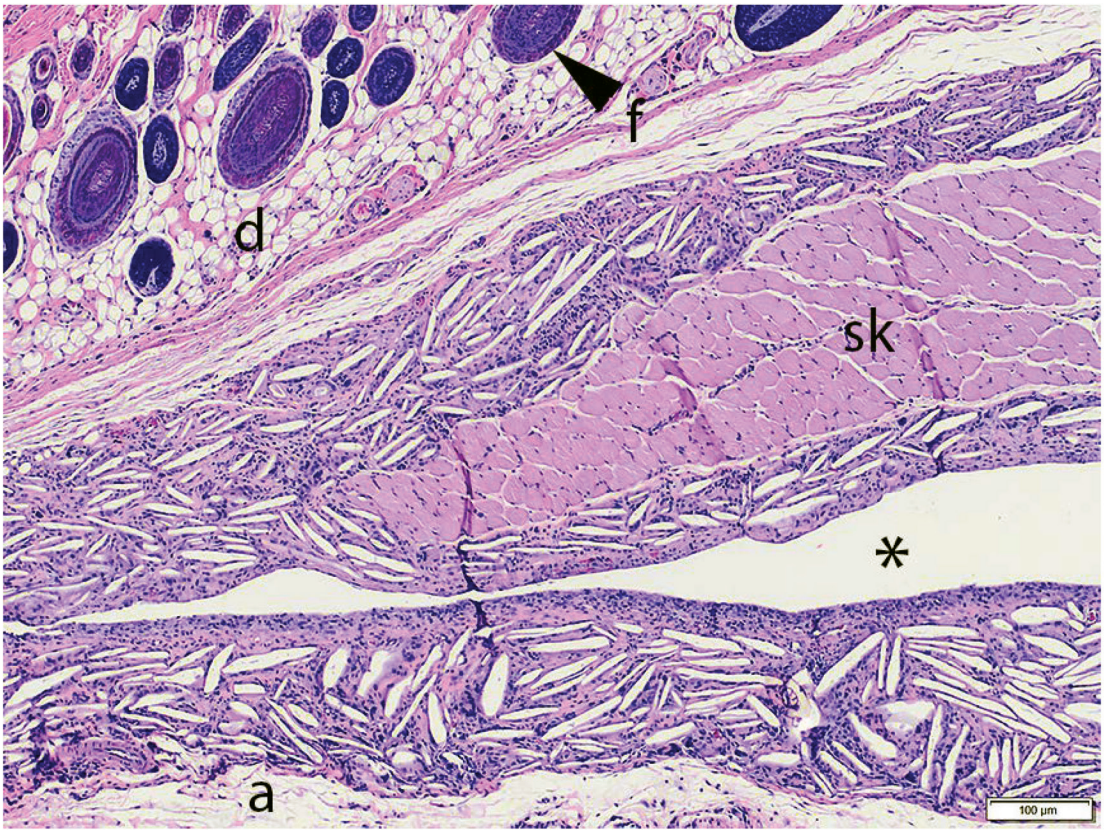

Figure 4. Skin from a Sprague–Dawley rat is shown at 40× total magnification, H and E stain, on day 15 following SC administration of 1.3 mg/kg (high dose) extended-release buprenorphine (BUP-XR). Chronic granulomatous inflammation encircles a clear cavity (asterisk) in the subcutis at the injection site. Clear clefts and cavities within the inflammatory region appear typical of lipid material, which was removed during histologic processing. a = adventitia, d = dermis, f = hair follicle, sk = skeletal muscle (panniculus carnosus).

a range of BUP dosages and BUP plasma levels, including in rats and mice.[7,9] On balance, the results of our study indicate that a single BUP-XR treatment at either the high or low dose can reliably provide plasma levels of buprenorphine that are reported in the literature to be therapeutically relevant in rats for up to 72 h. A repeat-dose indication for the approved label dose of 0.65 mg/kg provides the option for longer analgesia in models with extended convalescence periods (Ethiqa XR prescribing information). When the study results are considered as the basis for dose-determination of a commercial BUP-XR preparation, the 0.65 mg/kg dose would be suitable for most clinical applications in rats. Preference for the 0.65 mg/kg dose is also related to reducing the potential for adverse effects, which were minimal in low dose rats in our study.

The study found significant sex differences in plasma BUP concentrations, with female rats having lower mean values than did males at all time points for both BUP-XR doses (Figures 1 and 2) and a significantly lower AUC ($P = 0.010$) for each dose. Sex differences in opioid effects have been widely observed, including in rats and humans,[17,27] with differences in both pharmacodynamics and pharmacokinetics. In rats and mice, morphine administered systemically appears to be more effective in males than in females in a variety of pain models.[17] Although the effectiveness of opioids in pain relief appears to be in part due to differences in opioid receptors and sex differences in metabolism, both of these are affected by sex hormone levels.[17]

These data imply that in clinical settings, analgesia is less certain in females than in males, and that BUP-XR redosing, multimodal analgesia, and higher doses of BUP-XR should be considered depending on sex and the procedure being performed. The distinction in PK response of male and female rats is a key finding of our study that to our knowledge has not been reported previously for SR or extended release BUP agents. Possible sex differences in opioid responsiveness might mitigate the lower plasma levels in females. A unique feature of buprenorphine in humans is its relative mμ- (partial agonist) and κ- (antagonist) opioid receptor specificity, which may give it preferentially higher activity in women.[15,27] These observations support the further investigation of sex-specific responses to BUP-XR in rats.

A minimum of serious or prevalent adverse effects in treated rats was an encouraging outcome of the study. Minimal, transient weight loss in a minority of (3 of 24) rats was an indication of the essential safety of BUP-XR at either dose. The ANOVA analysis of the weight data did not reveal any statistically significant effects between dose group or sex for the change in weight from day 1 to day 8. However, a statistically significant difference was found for an effect of sex for the difference between day 1 and day 15, which reflected a larger weight gain by male rats, as is expected in rats of this age (male rats gained an average of 62 grams compared with the average weight gain of 23 grams for the female rats.). This contrasted with previous studies in which weight loss due to decreased food intake was common after treatment with various BUP-SR and non-SR formulations.[2,8,14] Pica behavior, a common side effect of BUP treatment in rodents,[3,8,19,24] was not observed, although signs of excessive grooming occurred in some high dose rats. The low number of adverse effects after treatment could be due to the diffusion-limiting effect of the lipid-encapsulated BUP formulation. Another study found that the linear plasma BUP

levels produced by SR agents avoid the peak-and-valley pattern that occurs with repeated dosing of conventional, non-SR formulations, which allow rapid exposure that may contribute to adverse effects at peak levels.[8] Absence of adverse events extended to necropsy findings in that treatment-related gross lesions or abnormalities were not found. This is comparable to results of a previous study of BUP-SR formulations.[24]

The forelimb skin irritation that occurred in several rats in the high dose group was not seen in low dose groups, suggesting that this could be a dose-related event caused by excessive grooming. Injection-site tissue reactions occurred in approximately half of the rats, an incidence that is typical of BUP-SR formulations containing polymer-encapsulated BUP.[3,8,19] Forelimb irritation, sores, scabs and alopecia in high dose rats were likely related to treatment because these signs are indicative of excessive grooming or pica, which were found to be BUP side effects in a previous study.[1] Mild to moderate injection-site granulomatous inflammation and minimal lymphocytic infiltrates observed in some of the test animals are an expected reaction to SC injection of material designed to persist in situ. The pronounced injection-site inflammation seen after SC administration of the BUP-XR high dose was not seen in rats given the 0.65 mg/kg dose. Forelimb lameness may have been due to manual restraint during blood collection.

Urinary abnormalities seen in some rats could also be treatment related because intermittent urinary retention is a known BUP side effect.[11] Other effects that developed after treatment were not considered by the pathologist to be related to BUP-XR treatment.

A limitation of the study was the small size of the test groups. Nonetheless, the mean AUC after BUP-XR administration was statistically consistent with a linear dose response.

An ethical imperative requires the control of pain in research animals. To that end, short acting nonextended release opioids are useful and effective analgesics but have practical limitations associated with repeat dosing. These include the adverse impact of frequent handling of test animals, increased personnel time, variable degrees of analgesia, and the potential for inadvertently influencing in vivo test results. The results of the current study indicate that plasma levels of BUP reported in the literature to be therapeutically relevant for analgesia lasting up to 72 h in rats can be rapidly achieved after a single SC dose of a novel BUP-XR formulation dosed at either 0.65 or 1.30 mg/kg. This approach provides the dual benefit of improved animal welfare and the potential for more consistent experimental outcomes when rat models are used.

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Conflict of Interest Statement

B Levinson and S Leary are affiliated with Fidelis Pharmaceuticals, manufacturer of Ethiqa XR (BUP-XR). The BUP-XR used in this study was provided by Fidelis, which also sponsored this work.

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