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ORIGINAL ARTICLE

Single-dose pharmacokinetics and bioavailability of a novel extended duration transdermal buprenorphine solution in cats

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Abstract

A novel transdermal buprenorphine solution (TBS) was developed for evaluation in order to make available an extended duration opioid analgesic for cats. Healthy adult cats were administered a single TBS dose of 10 mg (1.57–4.35 mg/kg), 30 mg (4.72–13.0 mg/kg), or 50 mg (7.87–21.7 mg/kg) (4 cats per group) applied topically to the unclipped dorsal cervical skin and plasma buprenorphine concentrations were evaluated through 7 days. To determine the absolute bioavailability of TBS, healthy cats were administered single TBS dose of 20 mg (3.33–4.76 mg/kg) or 0.05 mg (0.008–0.011 mg/kg) IV buprenorphine (6 cats per group). The mean ± standard deviation maximum plasma buprenorphine concentrations (C_{max}) were 10.5 ± 6.28, 18.6 ± 8.68, and 22.5 ± 4.47 ng/ml following 10, 30, and 50 mg doses, respectively, with the time of C_{max} occurrence (t_{max}) typically occurring at 2–12 h post-dosing. Mean plasma buprenorphine terminal half-lives ranged between 78.3 and 91.2 h. Increasing the dose threefold and fivefold from the 10 mg dose increased the exposure by 2.8- and 3.6-fold, respectively, indicating that plasma buprenorphine exposure increased in a less than proportional manner at doses >30 mg. Transient sedation, mydriasis, and euphoria were observed within 4 h post-dosing. Mean rectal temperatures were increased 0.6–0.9°C greater than baseline (37.4–37.8°C) through 168 h post-dosing. The absolute bioavailability was 16.0% (90% CI: [11.8%–21.7%]). Flip-flop pharmacokinetics were observed with a terminal elimination half-life of 0.82 ± 0.13 and 64.9 ± 15.0 h for IV buprenorphine and 20 mg of TBS, respectively. A single administration of TBS over a range of doses resulted in extended plasma buprenorphine concentrations and opioid physiological and behavioral effects.

KEYWORDS
analgesia, buprenorphine, cat, pharmacokinetics, transdermal

1 | INTRODUCTION

The medical benefits of integrating opioids into pain management approaches in cats have been established (Bortolami & Love, 2015) and the evidence for buprenorphine reviewed (Bortolami & Love, 2015; Steagall et al., 2014). Two pharmaceutical buprenorphine formulations have been approved for use in cats. The first, a low-concentration buprenorphine solution (0.3 mg/ml) was approved for use as IM and IV injection (0.01–0.02 mg/kg) in 1995 in the United Kingdom (UK) by the Veterinary Medical Directorate "Vetergesic; Ceva Animal Health"), and later by several other regulatory jurisdictions. It has not been approved by the Food and Drug Administration in the United States (US) to date. Parenteral injections of this formulation in cats have a short duration of action, possibly as short as 4 h,

particularly in the absence of NSAIDs (Steagall et al., 2009) and per the label, repeated injections are limited to a single injection 1–2 h following the first. Extra-label subcutaneous administrations are not effective and not recommended (Steagall et al., 2014).

A second buprenorphine solution was approved for use in cats limited to the United States in 2014 ('Simbadol; Zoetis'). It is a high-concentration injectable solution (1.8 mg/ml) developed to extend the analgesic duration of action compared to the low-concentration solution. A single SC dose (0.24 mg/kg) has an approximate 24-h analgesic duration (Doodnaught et al., 2017, 2018; Sramek et al., 2015). Whereas this extends the single injection duration of action compared to the low-concentration formulation, prolonging analgesia beyond the day of surgery requires repeated daily injections. However, repeated injections are limited to a total of three per the label and the product cannot be dispensed to owners for home injection, limiting the practicality of extending the duration beyond in-hospital use.

Other investigational methods to extend the duration of action of injectable solutions have been evaluated in cats. A sustained-release injectable buprenorphine extended the analgesic duration of action for several days in cats (Catbagan et al., 2011) but the effectiveness of this candidate product has not been adequately studied (Steagall et al., 2014). A more recent evaluation of an investigational liposomal encapsulated formulation administered subcutaneously to cats extended the duration of plasma buprenorphine over days compared to IV buprenorphine (Johnson et al., 2017). Neither formulation has been systematically developed, and compounded, unapproved products, are not a practical solution for wide clinical acceptance.

Extra-label and compounded formulations of buprenorphine have been evaluated to extend clinical options in cats, including oral transmucosal (OTM) and transdermal (TD) delivery. Buprenorphine has low molecular weight of 467.6, compact molecular structure, high lipid solubility, and adequate water solubility (Johnson et al., 2005), properties that render it ideal for OTM and TD delivery (Berti & Lipsky, 1995). The idea to evaluate OTM buprenorphine in cats originated from the successful use of sublingual transmucosal tablets in humans that were approved in 1980 ("Temgesic Sublingual Tablets; Indivior UK Limited"), and later, a sublingual film ("Suboxone sublingual film; Indivior UK Limited"). There are no approved OTM formulations for use in cats. Administration via this route utilizes compounded products made from injectable buprenorphine solution or sublingual tablets. In addition to variable absorption of compounded formulations (Gulledge et al., 2018), a single OTM dose has a limited duration of action similar to parenteral injections (Steagall et al., 2014). Attempts to extend the duration of the OTM route by extra-label administration of the full labeled dosage (0.24 mg/kg) of the high-concentration solution was no different compared to a single OTM administration (0.02 mg/kg) from a low-concentration formulation (0.3 mg/ml) (Doodnaught et al., 2018). When the high-concentration formulation was administered OTM at half the labeled dosage (0.12 mg/kg), thermal threshold differences were limited to 8 h compared to saline (Doodnaught et al., 2017).

For use in humans, transdermal matrix buprenorphine patches with an extended duration of action have been approved in Europe ("Transtec; Napp Pharmaceuticals") and the United States ("Butrans Transdermal System; Purdue Pharma L.P.") and are available in strengths designed to deliver zero-order TD buprenorphine release profile specified by the nominal rate identified on the patch ranging from 5 to 70 μg/h. When a 35 μg/h patch was applied to the clipped thorax of cats in a laboratory study, there was no significant change in thermal thresholds despite a slow rise in plasma buprenorphine concentrations (Murrell et al., 2007). Moreover, poor patch adhesion was observed whereby patches fell off in four of the six cats, and although they were quickly reattached, this could present a practical safety hazard to owners and cats in a clinical or home setting.

In addition to a limited duration of action, the need for compounding in some circumstances, and poor patch adhesion, current buprenorphine pharmaceutical formulations are accompanied with other medical and practical limits for use in cats. Repeat administrations of low- or high-concentration solution by parenteral or OTM routes result in peak-and-trough plasma concentrations, potentially allowing breakthrough pain near the end-of-dosing interval, when cats may be most vulnerable during unobserved periods. Moreover, parenteral injections are potentially painful and difficult to administer, contributing to stress and fear in both cats and medical personnel (Riemer et al., 2021). Finally, although extra-label use of OTM buprenorphine syringes dispensed to owners for repeated administrations at home may extend the analgesic duration and eliminate injection pain and stress, it does not alleviate the legal responsibility of dispensing buprenorphine, a Class III controlled substance in the United States (Kukanich & Papich, 2009).

To overcome the limitations of current buprenorphine formulations for use in cats, a novel, non-aqueous transdermal buprenorphine solution with a permeation enhancer intended for topical application has been developed (Zorbium™, Elanco US Inc., NADA 141-547). The objective of this initial product development study was to evaluate the PK and bioavailability of transdermal buprenorphine solution (TBS) across a range of doses in a laboratory study in cats following a single topical administration.

2 | MATERIALS AND METHODS

2.1 | Test article

The study was conducted in two phases. For the first PK phase of the study, TBS was formulated to contain 25 mg/ml buprenorphine (calculated as the free base), 5% w/v (50 mg/ml) padimate O as a permeation enhancer, and ethanol. To prepare the formulation, buprenorphine HCl (Spectrum Chemical Mfg. Corp.) was dissolved in a small amount of ethanol (Sigma-Aldrich) and padimate O (Sigma-Aldrich) was added and mixed. The formulation was brought to volume with ethanol and aliquoted into 10 ml amber glass vials sealed with a rubber stopper and aluminum crimp-top until use. In the second bioavailability phase of the study, the formulation was prepared

in an identical manner, but to a final buprenorphine concentration of 20 mg/ml. The control article for the bioavailability portion of the study was buprenorphine hydrochloride injectable solution (Buprenex, Reckitt Benckiser Pharmaceuticals, Inc.).

2.2 | Animals

All procedures were approved by the local Institutional Animal Care and Use Committee. For the first PK phase of the study, 12 adult domestic shorthaired cats (7 castrated males and 5 intact females) ranging in age from 1 to 4 years and weighing 2.35 to 6.35 kg were used. For the second bioavailability phase of the study, 12 adult domestic shorthaired male cats (6 castrated and 6 intact) ranging in age from 9 to 13 months and weighing 4.20 to 6.35 kg were used. Complete physical examinations were conducted on all cats prior to randomization to ensure animal health. Cats were individually housed in cages with solid stainless-steel walls on 5 sides (back, bottom, top, and 2 sides). Cage fronts served as the door and were composed of a welded grid of steel dowels. Water was provided ad libitum and a complete dry feline diet was provided in appropriate amounts to assure animal health. An alternating approximate 12-h light/dark cycle was maintained. Ventilation rates in the animal facility averaged approximately 10 complete air changes per hour with the temperature controlled between 18 and 29°C.

2.3 | Study design

For the first PK phase of the study, animals were randomized to receive a single 10, 30, or 50 mg (n = 4/group) dose of TBS. Randomization was blocked by bodyweight to maintain balance across dose groups. Animals were not fasted prior to treatment administration. Transdermal buprenorphine solution was administered topically to the unclipped skin on the dorsal cervical region (base of the skull) using the tip of a needleless syringe. The syringe tip was placed directly onto the skin at the application site, and the entire dose volume was administered at a single location without moving the syringe. The volumes of TBS administered were 0.4, 1.2, and 2 ml in the 10, 30, or 50 mg dose group, respectively. The cats were gently restrained for 2 min post-dosing to prevent the cat from shaking or grooming while the solution dried. Blood samples (~1 ml/sample) for plasma buprenorphine assay were collected from all cats prior to dosing and 2, 4, 12, 24, 48, 72, and 168 h post-dosing. Samples were collected by jugular venipuncture using a syringe and needle and immediately transferred into K_2EDTA tubes and placed on ice until plasma processing by centrifugation at 4°C for 15 min at 1500 *g*. Plasma samples were stored frozen at −20°C or below until analysis. Animal behavioral effects and mydriasis (0 = no, 1 = yes) were assessed, and rectal body temperatures were measured prior to dose administration and at 2, 4, 12, 24, 48, 72, and 168 h post-dosing. Behavior was scored on a 5-point scale (Appendix 1) as: 0—Normal, 1—Sedate, 2—Euphoric, 3—Mildly Dysphoric, or 4—Dysphoric.

For the second phase of the PK study, animals were randomized to receive a single 20 mg dose of TBS (1 ml) or 0.05 mg IV buprenorphine (n = 6/group). Randomization was blocked by bodyweight to maintain balance across dose groups. Animals were not fasted prior to treatment administration. Transdermal buprenorphine solution was administered topically in the same manner as in the first phase of the study, *that is*, on the dorsal cervical region. Injectable buprenorphine was administered IV as a bolus. To assure IV drug delivery, a temporary cephalic vein catheter was placed in each cat under sedation with 40 μg/kg IM dexmedetomidine hydrochloride (Dexdomitor, Zoetis Inc.). Sedation was reversed after catheter placement and prior to buprenorphine injection by administering 0.2 mg/kg IM atipamezole (Antisedan, Zoetis Inc.). Following IV administration of buprenorphine, the catheters were flushed with sterile saline and removed from the animals. Blood samples (~2 ml/samples) for plasma buprenorphine assay were collected from TBS-treated cats prior to dosing and 1, 2, 4, 12, 24, 48, 96, 168, and 240 h post-dosing and from IV buprenorphine cats prior to dosing and at 5 and 15 min, 1, 2, 4, 12, and 24 h post-dosing. Samples were collected and stored as described in the first phase of the study.

2.4 | Sample analysis

For the first PK phase of the study, plasma samples were analyzed for buprenorphine concentration using a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method. A 100 μg/ml stock solution of buprenorphine HCl (Cerilliant®) was diluted into 50:50 methanol (Honeywell Burdick & Jackson):water to create working solution standards ranging from 0.500 to 1250 ng/ml and quality control (QC) working solutions of 3.00, 375, and 1000 ng/ml. Similarly, a 100 μg/ml stock solution of the internal standard (IS) buprenorphine-d_4 (Cerilliant®) was diluted into acetonitrile to create a working IS solution of 10 ng/ml. Calibration and QC standards were then prepared by adding 10.0 μl of the appropriate working solution standards or QC working solutions to 50.0 μl of control blank feline plasma. Likewise 10.0 μl of 50:50 methanol:water was added to 50.0 μl of all study samples, blanks, and zero controls. Subsequently, 250 μl of the IS working solution was added to all calibration standard, QC, study, and zero control samples; and 250 μl of acetonitrile was added to all blanks. Samples were vortex-mixed for 2 min and then centrifuged for 10 min. Fifty (50.0) μl of the supernatant was transferred to a 96-well elution plate containing 250 μl of reverse osmosis water and vortex-mixed for 2 min. Water diluted samples were quantified using an API 5000™ triple quadrupole mass spectrometer equipped with TurboIonSpray™ interface (AB SCIEX) with peak area integration conducted using Analyst Software v 1.5.1 (AB SCIEX) data acquisition system. HPLC separation was achieved using a Phenomenex Gemini C18 (50 × 3 mm, 5 μm particle size) column (Phenomenex) with the flow rate set at 0.700 ml/min and a column temperature of 30°C. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B consisted of 0.11% formic acid in acetonitrile. The mobile phase gradient started at 10% mobile phase B

from 0.0 to 0.5 min, switched from 10% to 80% mobile phase B from 0.5 to 2.0 min, and switched back from 80% to 10% mobile phase B from 3.0 to 3.1 min. The injection volume was 5 µl and mass spectrometer detection was conducted using positive ionization mode and monitoring of the transitions 468.5 m/z → 396.3 m/z for buprenorphine and 472.5 m/z → 400.3 m/z for the IS buprenorphine-d_4. Both analytes typically eluted from the column at 1.82 min. Standard curves were determined using linear regression with $1/x^2$ weighting using Excel (Version 11, Microsoft Corporation), where x is the nominal sample concentration, and had typical squared correlation coefficient (R^2) values of .9977–.9993. All concentration calculations were based on the peak area ratios of buprenorphine to the IS. The calibration concentration range for buprenorphine was 0.100–250 ng/ml with a lower limit of quantification (LLOQ) of 0.100 ng/ml. The intra- and inter-assay precision (i.e., coefficient of variation) were ≤5.95% and the accuracy (i.e., relative error) ranged from 0.00% to 2.67%.

A similar validated LC-MS/MS method was used for the second bioavailability phase of the study with modification. The calibration concentration range for buprenorphine was 0.200–100 ng/ml with a lower limit of quantification (LLOQ) of 0.200 ng/ml. The buprenorphine metabolite norbuprenorphine was demonstrated to not interfere with the quantification of plasma buprenorphine. The intra- and inter-assay precision (i.e., coefficient of variation) were ≤8.2% and the accuracy (i.e., relative error) ranged from −4.3% to 7.5%. This method was conducted and reported in compliance with the US Food and Drug Administration (FDA), Department of Health and Human Services, Good Laboratory Practices (GLP) (Title 21 Code of Federal Regulations Part 58).

2.5 | Pharmacokinetic analysis

Plasma buprenorphine concentrations <LLOQ were excluded from the summary statistic calculations. PK parameters were calculated for each subject using noncompartmental PK analysis methods with Phoenix™ WinNonlin® Version 6.2 (Build 6.2.0.495, Pharsight® - A Certara Company). The linear trapezoidal rule was used for the area under the plasma concentration-time curve (AUC) calculations. The absolute bioavailability (F) of TBS was estimated using least squares means as the ratio of the geometric means of the bodyweight dosage adjusted AUCs across individual cats.

3 | RESULTS

For the first phase of the study, the range of doses administered on bodyweight basis in the 10, 30, and 50 mg groups were 1.57–4.35, 4.72–13.03, and 7.87–21.73 mg/kg, respectively. Three plasma buprenorphine concentrations were considered outliers. Two concentrations from the 30 mg group were excluded: a 12-h sample was 54.6 ng/ml and a 48-h sample was 72.6 ng/ml. A single concentration excluded from the 24-h time point in the 10 mg treatment group

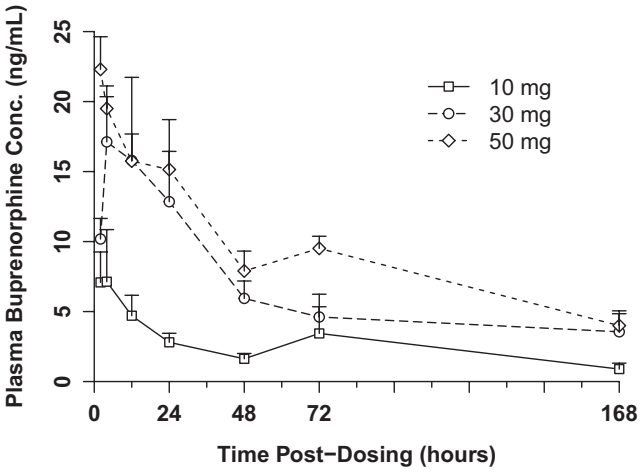

FIGURE 1 Mean plasma buprenorphine concentrations following a single TBS administration of 10, 30, or 50 mg ($n = 4$ cats per treatment group). Bars indicate the standard error

that was 24.8 ng/ml. The reason for these outlier observations was not determined by study audit, but the measured concentrations were confirmed by re-assay. A sensitivity analysis was subsequently conducted on the plasma buprenorphine concentrations by removing these three observations and then re-calculating the plasma concentration summary statistics and the PK analysis. There was no difference with the outliers removed and therefore the samples remained excluded from the analysis below.

For the first phase of the study, plasma buprenorphine reached peak mean concentrations between 2 and 4 h post-dosing and all samples remained above the LLOQ through 168 h post-dosing (Figure 1). The mean plasma buprenorphine concentrations in the 50 mg TBS group were occasionally marginally higher than, or the same as, those in the 30 mg dose group. The mean ± standard deviation C_{max} values were 10.5 ± 6.28, 18.6 ± 8.68, and 22.5 ± 4.47 ng/ml following 10, 30, and 50 mg TBS doses, respectively (Table 1). The time of C_{max} occurrence (t_{max}) ranged from 2 to 12 h, except for a single value of 72 h in the 10 mg dose group. The mean terminal half-lives ($t½$) ranged from 78.3 to 91.2 h. The mean percentages of AUC extrapolated ranged from 21.8% to 24.9% across dose groups. The mean ± standard deviation area under the curve from time 0 to infinity ($AUC_{0-\infty}$) were 578 ± 348, 1590 ± 1180, and 2070 ± 499 h ng/ml following 10, 30, and 50 mg doses, respectively. There was 2.8- and 3.6-fold increase in $AUC_{0-\infty}$ in the 30 and 50 mg dose group, respectively, compared to the 10 mg group.

Transient sedation and euphoria were observed beginning within 2 h post-dosing (Figure 2). Twenty-five to 50% of cats were sedated (behavioral score = 1) for 24 h in the 10 mg group. In the 30 and 50 mg groups, sedation was observed in 25 to 50% of cats for 48 h, with no effects observed after 72 h. Euphoria (behavioral score = 2) was observed in 25 to 75% of cats in all dose groups through 24 h with no euphoria observed beyond 72 h. Neither mild dysphoria (behavioral score = 3) nor dysphoria (behavioral score = 4) was observed at any time in the study. Mean rectal body temperatures peaked at 12 h post-dosing and appeared to be greater in the 30 and

TABLE 1 Pharmacokinetic by dose following administration of 10, 30, or 50 mg of TBS ($n = 4$ cats per treatment group). Results presented as mean ± SD (range)

Dose	C_{max} (ng/ml)	t_{max} (h)	$AUC_{0\text{-LLOQ}}$ (h ng/ml)	$t_{½}$ (h)	$AUC_{0\text{-}\infty}$ (h ng/ml)	AUC Extrapolated (%)	C_{max}/D (ng/ml/mg)	$AUC_{0\text{-}\infty}$/D (h ng/ml/mg)
10 mg	10.5 ± 6.28 (3.02–18.1)	20.0 ± 34.7 (2–72)	448 ± 262 (156–718)	91.2 ± 22.9 (60.8–114)	578 ± 348 (218–967)	21.8 ± 13.4 (6.33–36.6)	1.05 ± 0.628 (0.302–1.81)	57.8 ± 34.8 (21.8–96.7)
30 mg	18.6 ± 8.68 (10.6–27.6)	5.50 ± 4.43 (2–12)	1120 ± 685 (570–2120)	78.3 ± 22.2 (51.1–105)	1590 ± 1180 (658–3310)	24.3 ± 9.74 (13.4–36.0)	0.621 ± 0.289 (0.353–0.920)	53.1 ± 39.4 (21.9–110)
50 mg	22.5 ± 4.47 (19.5–29.0)	4.50 ± 5.00 (2–12)	1520 ± 214 (1220–1690)	90.0 ± 18.5 (69.3–114)	2070 ± 499 (1500–2710)	24.9 ± 9.70 (16.0–37.8)	0.450 ± 0.090 (0.390–0.580)	41.4 ± 9.99 (30.0–54.2)

Note: $AUC_{0\text{-LLOQ}}$: AUC from time 0 to the time of the last sample at or above the LLOQ; C_{max}/D: Dosage normalized C_{max}

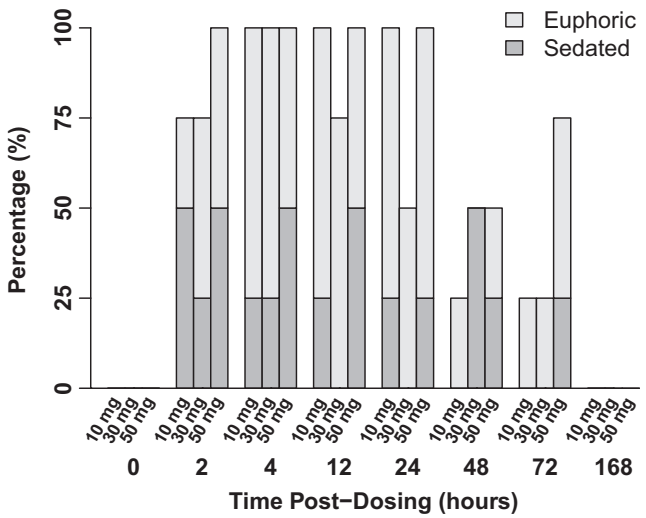

FIGURE 2 Percentage of cats sedated and euphoric by time following a single TBS administration of 10, 30, or 50 mg ($n = 4$ cats per treatment group)

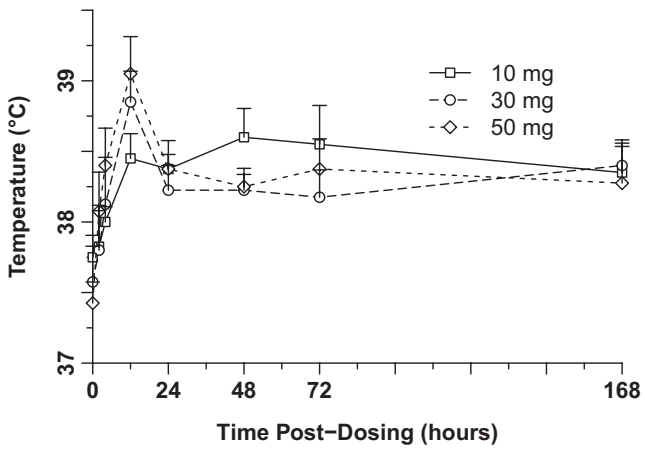

FIGURE 3 Mean rectal body temperatures following a single TBS administration of 10, 30, or 50 mg ($n = 4$ cats per treatment group). Bars indicate the standard error

50 mg TBS dose groups (38.9 and 39.1°C, respectively) than in the 10 mg dose group (38.5°C) (Figure 3). The mean temperatures remained from 0.6–0.9°C greater than baseline (37.4–37.8°C) through 168 h post-dosing. Mydriasis was observed in 75% to 100% of cats in each dose group between 4 and 12 h post-dosing. No mydriasis was observed in any cats in the 10 and 30 mg TBS dose groups from 48 h post-doing onward. Mydriasis was observed in at least 50% of cats administered 50 mg TBS through 72 h post-dosing. No mydriasis was observed in any cats beyond the 72 h observation.

For the second phase of the study, on a bodyweight basis, the mean (range) buprenorphine dosages following 0.05 mg IV and 20 mg TBS administration were 0.00972 (0.00787–0.0112) and 3.95 (3.33–4.76) mg/kg, respectively (Table 2). Plasma buprenorphine concentrations from the IV group rapidly decreased from a mean of 13.6 ng/ml at 5 min post-dosing to 0.231 ng/ml by 4 h post-dosing and were <LLOQ beyond 4 h (Figure 4). In contrast, the mean plasma buprenorphine concentrations from the TBS group peaked at 1 h and gradually decreased (Figure 4); mean concentrations were 11.6, 7.11, 1.86, and 0.513 ng/ml at 1, 24, 96, and 240 h post-dosing, respectively. The C_{max} and t_{max} following TBS administration were 15.1 ± 7.25 ng/ml and 7.33 ± 9.16 h, respectively, and the initial concentration (C_0) following IV administration was 18.4 ± 4.88 ng/ml. The clearance (Cl) following IV administration was 16.7 ± 4.28 ml/min kg. The $t½$ following IV and TBS administration were 0.82 ± 0.13 and 64.9 ± 15.0 h, respectively. The percent AUC extrapolated was <20% for all subjects. The estimated absolute bioavailability (F) of TBS was 16.0% (90% CI: [11.8%–21.7%]).

4 | DISCUSSION

The results from this study indicate that buprenorphine can be delivered through the skin of cats for an extended duration compared to IV or IM administration in potentially analgesic concentrations without the need for a device or patch. The analgesic concentration of buprenorphine has been proposed in cats. When PK and PD are co-examined, antinociception during the plasma buprenorphine decline phase is greater than that observed when plasma concentrations are rising demonstrating anticlockwise negative hysteresis (Murrell et al., 2007; Robertson et al., 2005; Steagall et al., 2013). The presumptive mechanism for this observation is a reflection of the time it takes for buprenorphine to distribute to its effect site (i.e., brain and spinal cord) and its high affinity and slow off time from opioid receptors (Steagall et al., 2013). In a PK-PD laboratory study

TABLE 2 Pharmacokinetics following administration of 20 mg of TBS or 0.05 mg/kg IV buprenorphine ($n = 6$ cats per treatment group). Results presented as mean (range) or estimate [90% CI]

Treatment	Dose (mg)	Dosage (mg/kg)	C_{max} or C_0[a] (ng/ml)	t_{max} (h)	$AUC_{0-\infty}$ (h ng/ml)	$t_{½}$ (h)	AUC Extrapolated (%)	Cl (ml/min kg)	F (%)
Transdermal	20	3.95 ± 0.520 (3.33–4.76)	15.1 ± 7.25 (4.82–25.6)	7.33 ± 9.16 (1–24)	668 ± 214 (470–1030)	64.9 ± 15.0 (39.1–85.7)	9.32 ± 4.63 (5.98–18.3)	-	16.0 [11.8–21.7]
Intravenous	0.05	0.00972 ± 0.00132 (0.00787–0.0112)	18.4 ± 4.88 (14.2–27.5)	-	9.99 ± 1.79 (8.11–13.1)	0.82 ± 0.13 (0.59–0.97)	3.75 ± 2.31 (2.29–8.34)	16.7 ± 4.28 (12.4–23.2)	-

[a]Calculated by back-extrapolation to time 0; $AUC_{0-\infty}$: AUC from time 0 to infinity.

FIGURE 4 Mean plasma buprenorphine concentrations following administration of 20 mg of TBS or 0.05 mg IV buprenorphine ($n = 6$ cats per treatment group). Bars indicate the standard error. Inset expands the *x*-axis over the first 4 h

with injectable buprenorphine solution, there was an anticlockwise negative hysteresis loop with an EC50 analgesic plasma concentration of 2.3 ng/ml calculated from the offset concentration (Taylor et al., 2016). Following topical application of a range of TBS doses in the present study, mean plasma buprenorphine concentrations exceeded 2.3 ng/ml at the 2-h sample time for all three doses suggesting rapid onset of action. Mean terminal half-lives ranged from 78.3 to 91.2 h across the 10, 30, and 50 mg TBS doses supporting extended duration. Mean buprenorphine concentrations exceeded 2.3 ng/ml through 168 h at the 30 and 50 mg doses and were above 2.3 ng/ml for 72 h at the 10 mg dose with the exception of the 48-h sampling point. Further supporting a pharmacological effect, the plasma buprenorphine concentrations were temporally associated with behavioral and physiological effects consistent with opioid exposure including transient sedation, euphoria, mydriasis, and increased rectal temperature.

The doses chosen to evaluate in this study were based on literature PK values and assumptions of bioavailability. Literature values for Cl (16.7 ml/min kg) were used following IV buprenorphine solution administration (Taylor et al., 2001). Assumed transdermal bioavailability was 25% based on fentanyl and buprenorphine bioavailability from transdermal patches in cats (Lee et al., 2000; Murrell et al., 2007). Using these inputs, to achieve a steady-state plasma buprenorphine concentration of 10 ng/ml, a 5 kg cat would require a zero-order input of 37.8 μg/h. When this rate is delivered over a 7-day period, the estimated topical dose would be 35 mg. To test a range of doses on both sides of this estimate, single topical doses of 10, 30, and 50 mg were evaluated. To calculate bioavailability of a possible clinical dose for further study, a single topical dose of 20 mg was evaluated in comparison with a 0.05 mg IV injection.

The results based on these assumptions were generally achieved. Mean plasma buprenorphine concentrations at sampling points from 2 to 72 h ranged from 1.63–8.3, 4.61–17.1, and 7.90–22.3 ng/ml following 10, 30, and 50 mg TBS administration, respectively. It

should be noted that a true steady state was not achieved, as plasma buprenorphine concentrations generally slowly declined following C_{max}. Bioavailability was 16% (12.4–23.5%) which was near the estimated target and similar to estimates of buprenorphine bioavailability from patches in cats (Murrell et al., 2007). From the bioavailability phase of the study, the calculated PK inputs from IV buprenorphine were replicated. Buprenorphine Cl was 16.7 ± 4.28 ml/min kg, similar to that reported (Johnson et al., 2017; Taylor et al., 2001). Although the mean *t½* following IV buprenorphine, 0.82 h (49 min), was shorter than the range of means reported from 4.39 to 12.3 h (Doodnaught et al., 2017; Hedges et al., 2014; Johnson et al., 2017; Robertson et al., 2005; Steagall et al., 2013; Taylor et al., 2001), it was similar to several individual animal results (Johnson et al., 2017) and that observed following lumbosacral epidural administration (Duke-Novakovski et al., 2011). The reason for these differences in reported half-lives is unknown and may have to do with relatively small sample sizes and/or differing bioanalytical methodology. The bioanalytical method utilized in the current study was validated per GLP standards. Regardless, the more robustly determined parameter of buprenorphine Cl was highly consistent across studies, confirming validity of the current study.

The skin poses a technical challenge in that it is normally an efficient barrier to exclude xenobiotics (Riviere & Papich, 2001). However, as demonstrated with the formula used in this study, permeation enhancers and solvents can promote flux of drugs across skin by disrupting the ordered structure of the intercellular lipids in the stratum corneum (El Maghraby et al., 2005; Morgan et al., 1998; Morgan et al., 1998), thereby overcoming the barrier nature of the skin. The deposition of buprenorphine into the skin as a depot is demonstrated by the observed flip-flop or absorption-dependent PK. In the plasma concentration-time curve, the downward portion of the curve is the result of decreased drug absorption rather than drug elimination mechanisms (Figure 1). This was confirmed in the bioavailability phase of the study. The *t½* following IV and TBS administration were 0.82 ± 0.13 and 64.9 ± 15.0 h, respectively (i.e., flip-flopped), whereby the much longer half-life in the TBS group was attributable to buprenorphine absorption from a depot in the skin.

The site selected to topically apply TBS was the unclipped dorsal cervical skin. The rationale for this site selection was to allow cats to be in a comfortable, stress-free conformation during application and to prevent cats from immediately grooming the application site. Other skin sites could result in different rates of absorption. A rank order of skin penetration by non-ionized compounds has been demonstrated. For example, in humans, the skin sites from greatest to least transdermal absorption is scrotal >forehead > axilla =scalp > back =abdomen > palm and plantar (Riviere & Papich, 2001). Site preferential absorption has not been systematically studied in cats.

Increasing the TBS dose threefold and fivefold from the 10 mg dose increased the area under the curve ($AUC_{0-\infty}$) 2.8- and 3.6-fold, respectively. The lack of dose proportionality in exposure is also illustrated by the declining mean dosage normalized AUCs ($AUC_{0-\infty}$/D) as the dose increased (Table 1). Due to the less than proportional increase in plasma buprenorphine exposure with increasing dose, larger doses of TBS may not result in substantially increased effectiveness and suggests an upper limit of buprenorphine absorption and flux. Across the first and second phase of the study, the TBS PK were largely consistent. The 20 mg TBS C_{max} of 15.1 ± 7.25 ng/ml in the second phase was between the 10 and 30 mg C_{max} values from the first phase of 10.5 and 18.6 ng/ml, respectively. Likewise, the $AUC_{0-\infty}$ at the 20 mg dose was between that of the 10 and 30 mg doses. The $t_{½}$ at 20 mg of 64.9 ± 15.0 h was less than that at 10 and 30 mg of 91.2 ± 22.9 and 78.3 ± 22.2 h, respectively; however, the ranges of $t_{½}$ broadly overlapped across all doses.

The observed physiological and behavioral effects confirm pharmacological actions from buprenorphine exposure. Sedation, euphoria, mydriasis, and increased rectal temperatures are well-described effects in cats following buprenorphine administration (Kukanich & Papich, 2009). Consistent with a potential analgesic duration of action of multiple days, the observed pharmacodynamic effects lasted for several days. Euphoria was present in at least 1 cat at all doses through 3 days post-dosing, as was mydriasis at the 50 mg dose. The observed modest increase in body temperature is known to occur with buprenorphine in cats, though it is not generally considered to be clinically relevant (Cannarozzo et al., 2021; Posner et al., 2010; Steagall et al., 2014). The mean temperatures remained 0.6–0.9°C greater than baseline (37.4–37.8°C) through 168 h post-dosing, but never reached ≥40.0°C in any individual cats.

Plasma buprenorphine concentrations and analgesia have been linked through direct observation of plasma concentrations and antinociception through PK-PD modeling and may serve as a guide in subsequent studies (Doodnaught et al., 2017; Hedges et al., 2014; Robertson et al., 2005; Steagall et al., 2013; Taylor et al., 2016). The mean t1/2 across all doses of TBS ranged from 78.3 to 91.2 h and a single dose could deliver 3 or more days of analgesia. To link the observed data to antinociception, laboratory studies such as thermal threshold studies to establish a PK-PD relationship will be necessary. Finally, randomized controlled clinical studies in client-owned cats undergoing surgeries will confirm modeled data given that there is a delay between the plasma buprenorphine concentrations and analgesic response (Robertson et al., 2005; Taylor et al., 2016) and poor correlations between plasma buprenorphine concentrations and analgesia have been reported in cats and other species (Boas & Villiger, 1985; Murrell et al., 2007; Nolan et al., 1987).

5 | CONCLUSION

These results indicate that the dermal barrier to drug penetration can be overcome for buprenorphine through the use of a permeation enhancer and a rapidly evaporating solvent. Drug deposition into the skin as a depot following TBS administration was confirmed by flip-flop PK with a bioavailability similar to other transdermal products. A single administration of TBS resulted in plasma buprenorphine concentrations likely to provide analgesia for multiple days at all examined doses although further studies of TBS across a range of

doses are warranted to determine its analgesic efficacy. The product characteristics of TBS have the potential to overcome the limitations of other approved or compounded buprenorphine products used in cats including limited duration of action, the need for repeated administrations, dispensing controlled substances, end-of-dosing interval breakthrough pain, and offer the advantage of in-hospital, fear-free, stress-free administration, and prolonged duration of action.

AUTHOR CONTRIBUTION

KJF was involved in study design, study execution, data analysis, and manuscript preparation. CR was involved in study execution and manuscript preparation. KW led analytical method development and sample and data analysis. TL contributed to study design, data analysis, and manuscript preparation. TPC contributed to study design, data analysis, and manuscript writing. All authors have read and approved the final manuscript.

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ANIMAL WELFARE AND ETHICS STATEMENT

The authors confirm compliance to the ethical policies of the journal, as noted on the journal's author guidelines page. Prior to study initiation, an independent Institutional Animal Care and Use Committee reviewed and approved animal use protocol ETCR-11-0065, confirming compliance to United State Department of Agriculture animal welfare and ethics standards for the protection of animals used for scientific purposes. [Correction added on 26 August 2022, after first online publication: The Animal Welfare and Ethics Statement was included in this current version.]

CONFLICT OF INTEREST

All authors were paid employees (Freise, Lin, and Clark) or contractors (Reinemeyer and Warren) of Nexcyon Pharmaceuticals, Inc. at the time the study was conducted. One author (Clark) was a paid consultant of Elanco Animal Health at the time the manuscript was written, and one author (Lin) was a paid consultant of Nexcyon Pharmaceuticals, Inc. at the time the manuscript was written.

DATA AVAILABILITY STATEMENT

Submitted with manuscript.

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APPENDIX 1

Behavior Scale

Behavior was assessed using a 5-point scale as follows:

0—Normal

1—Sedated—Subdued and quiet; signs include sleeping, ventral tail curling, and purring; 6 less responsive to human interaction.

2—Euphoric—Exaggerated social and playful behavior; signs include meowing, rolling, 9 kneading with forepaws, play-biting, and rubbing its head and body on cage.

3—Mildly Dysphoric—State of uneasiness and discord; signs include absent staring, 12 hyper-responsiveness, swaying, and/or vocalization, and may be accompanied by 13 increased locomotor activity; no overt signs of fear or disorientation, and no signs of 14 aggression; may initially appear sedated, but then startle suddenly (i.e., hyper-15 responsive).

4—Dysphoric—State of anxiety or agitation; signs include staring at objects that are not present, hyper-responsiveness, sudden movements, and/or vocalization, and may be accompanied by increased locomotor activity; cats are obviously disoriented or fearful, may become aggressive.