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Pharmacokinetics and dose selection of a novel, long-acting transdermal fentanyl solution in healthy laboratory Beagles

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A novel, transdermal fentanyl solution (TFS) was developed that delivers sustained concentrations of fentanyl for days following a single application. The pharmacokinetics following a single topical dose was examined. Eighteen adult Beagle dogs were divided into three groups of six dogs (3M, 3F). Each group was administered a single dose of 1.3 (25), 2.6 (50), or 5.2 mg/kg (100 μL/kg) of TFS. The dose was applied to the clipped, ventral abdominal skin using a 1-mL tuberculin syringe. Immediately following dosing, collars were placed on each dog through 72 h to prevent direct licking of the application site. Serial jugular venous blood samples were collected at 0 (predosing), 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 144, 168, 240, 336, 408, and 504 h after dosing and assayed for plasma fentanyl concentration. Fentanyl was rapidly detected following application with a mean absorption lag time (t_{lag}) of 0.333 h in the 1.3 mg/kg group and 0 in the other two groups. The mean C_{max} increased with dose and were 2.28, 2.67, and 4.71 ng/mL in the 1.3, 2.6 and 5.2 mg/kg dose groups, respectively. Mean terminal half-lives were 53.7, 69.6, and 103 h in the 1.3, 2.6, and 5.2 mg/kg dose groups, respectively. The mean $AUC_{0\text{-}LLOQ}$ from lowest to highest dose groups were 157, 268, and 645 ng·h/mL and were dose proportional with a R^2 value of 0.9818. Adverse reactions were limited to the highest dose group and included sedation (four of six dogs) and decreased food and water intake (one dog). A dose of 2.6 mg/kg (50 μL/kg) is proposed for further development studies based on the lack of adverse events that were observed compared to the 5.2 mg/kg group and a more rapid onset of action and longer duration of action compared to the 1.3 mg/kg group.

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INTRODUCTION

Opioids are generally regarded as an important part of multimodal perioperative analgesia, especially for moderate to severe pain. In human health, the use of opioids during and after surgery for most soft tissue and orthopedic surgeries is considered the standard of care and are included in procedure-specific treatment algorithms. (Neugebauer *et al.*, 2007) In veterinary medicine, the use of opioids in dogs beyond the immediate postoperative period is prevented by inherent limitations of most opioids including poor oral bioavailability and rapid clearance. (Pascoe, 2000) As a result, opioid use is primarily limited to single or repeat perioperative parenteral injections or constant rate intravenous infusions (CRI) delivered during anesthesia.

Fentanyl is a potent, full μ-opioid receptor agonist having approximately 100-fold the analgesic potency of morphine (Adams, 2001). Poor oral bioavailability and rapid clearance have limited fentanyl's use primarily to perioperative parenteral doses. Following intravenous (IV) administration of fentanyl citrate to dogs, the elimination half-life has been reported to range from 0.76 to 6.0 h (Murphy *et al.*, 1983; Kyles *et al.*, 1996; Hughes & Nolan, 1999; Sano *et al.*, 2006). To overcome these limitations and prolong the therapeutic duration of action, variations in pharmaceutical delivery have been advanced for use in human health. For prolonged use to treat moderate to severe chronic pain in humans, patches intended as devices to deliver fentanyl transdermally have been developed (Janssen Pharmaceutica Products, 2005). Although specifically contra-

indicated for use for postoperative pain in humans because of respiratory depression, patches have been examined for extra-label use in dogs. There are multiple shortcomings associated with their use in dogs that include: 1.) the lack of regulatory approval (Janssen Pharmaceutica Products, 2005), 2.) slow onset of action (Hofmeister & Egger, 2004), 3.) problems associated with maintaining patch contact on skin (Riviere & Papich, 2001), 4.) variable delivery rate and extent (Kyles *et al.*, 1996; Mills *et al.*, 2004), 5.) potential inadvertent fentanyl exposure to the pet or pet owner (Schmiedt & Bjorling, 2007), 6.) severe adverse events in children that inadvertently ingest patches (Teske *et al.*, 2007), 7.) concern for proper control and disposal of used patches, 8.) the possibility of diversion and illicit patch use when the pet is discharged from the hospital, and 9.) lack of regulatory oversight and pharmacovigilance to track adverse events in dogs.

A product that overcomes the limitations of parenterally, orally or patch-delivered opioids would be beneficial to pain management in dogs. A rational option would be a transdermal method that extends the duration of action and the bioavailability of opioids without the use of a patch. As a delivery method, direct transdermal drug absorption encounters the barrier nature of the skin making it difficult for most drugs to be delivered by this route (Barry, 1983). Some pharmaceutical attempts to extend the delivery of fentanyl without a patch have not been successful; topically applied fentanyl in a pluronic lecithin organogel did not result in measurable plasma concentration in dogs (Krotscheck & Boothe, 2004). However, the use of novel penetration enhancers is a potential strategy to improve percutaneous absorption. Penetration enhancers may act by disrupting the packing of skin lipids and thus altering the barrier nature of the stratum corneum, by changing the partitioning behavior of the drug at the stratum corneum-viable epidermis interface or by affecting the thermodynamic activity of the drug (Barry, 1987; Beastall *et al.*, 1988). A candidate for use as a safe skin penetration enhancer is octyl salicylate (OS) (Morgan *et al.*, 1998a,b,c). When it is combined with fentanyl in a rapidly evaporating solvent, super-saturation of both OS and the fentanyl occur at the moment of drying, resulting in rapid dermal absorption and sequestration into the stratum corneum. In this study, a transdermal fentanyl solution (TFS) containing OS as a penetration enhancer and isopropanol as the rapidly evaporating solvent was examined when applied topically to dogs at three different doses.

MATERIALS AND METHODS

Investigational drug

The investigational drug was TFS[a]. It is a clear, colorless to light yellow solution that contains 5% w/v (50 mg/mL) fentanyl base, the skin penetration enhancer OS (octisalate and 2-ethylhexyl salicylate) at a concentration of 5% w/v (50 mg/mL) and isopropanol (*qs*).

[a]Recuvyra™ transdermal solution (fentanyl) (Nexcyon Pharmaceuticals Ltd, London, UK). LLOQ, lower limit of quantification.

Animals

Eighteen purpose-bred laboratory Beagles (nine males/nine females) weighing 7.2 ± 0.82 (mean ± SD) kg were randomly selected for inclusion. Dogs were individually housed, fed a commercial dry food formula, and allowed *ad libitum* access to water. Dog were acclimated to the study room and cages 2 weeks prior to dose administrations. Bite-Not® collars (Bite Not Products, Inc., San Francisco, CA, USA) were used to prevent direct licking of the dosing site on the ventral abdominal area. Dogs were acclimated to the collars for approximately 1–2 h daily for 10 days to prior to dosing. Food was withheld overnight prior to dose application and continued until approximately 1 h post dose. All procedures were approved by the local Institutional Animal Care and Use Committee.

Study design

In groups of six dogs (three males/three females), 1.3 (25), 2.6 (50), or 5.2 mg/kg (100 μL/kg) of TFS was applied as a single dose to the ventral abdomen from approximately the umbilicus caudally. Prior to dose administration, the hair of the ventral abdomen was clipped. The calculated dose was drawn up into a 1-mL tuberculin syringe and applied by placing the syringe opening against the skin and depressing the syringe plunger while moving the tip in a serpentine manner to spread the small volume of liquid evenly. Animals were restrained for approximately 2 minutes following dose administration to allow the solution to dry. From prior to dosing until 72 h after dosing, Bite-not® collars were used to prevent direct licking of the dosing site on the ventral abdominal area. Animals were observed for sedation pre-dosing, 1 and 8 hours post-dosing, and daily thereafter through 144 hours (6 days) post-dosing. The sedation assessment was based on the clinical integration of animal behavior and multiple physiological variables. Serial jugular venous blood samples were collected via venipuncture at 0 (predosing), 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 144, 168, 240, 336, 408, and 504 h after dosing into sodium heparin blood collection tubes. Plasma was harvested from the blood samples by centrifugation at 1500 g for 10 min at 4 °C and stored at −70 °C until analysis.

Sample analysis

Plasma samples were analyzed for fentanyl concentration using liquid chromatography and tandem mass spectrometry (LC-MS/MS). Control dog plasma (Bioreclamation Inc., Hicksville, NY, USA) was serially diluted with a fentanyl (Sigma, St. Louis, MO, USA) working solution (100 ng/mL in 50:50 methanol [Fisher Scientific, Pittsburgh, PA, USA]/water [Fisher Scientific]) to create standard curve samples ranging from 0.025 to 10 ng/mL (7 calibration standards) and quality control (QC) samples at concentrations

of 0.065, 5, and 9 ng/mL. Fentanyl-d5 (Cerilliant®; Round Rock, TX, USA) was used as the internal standard (IS) (20 ng/mL in 50:50 methanol/water working solution). Two hundred microliters of sample, standard, QC, or control blank was aliquoted into 2-mL polypropylene microcentrifuge tubes (Fisherbrand; Fisher Scientific), and 20 μL of the IS working solution was added to all tubes except for the control blanks. Samples were vortexed and extraction conducted by protein precipitation using 20 μL of formic acid (Sigma-Aldrich, St. Louis, MO, USA) followed by 1.0 mL of acetonitrile (Fisher Scientific). Following protein precipitation, the samples were centrifuged at 20,000 *g* for 10 min and the supernatant transferred to clean 2-mL polypropylene microcentrifuge tubes. The supernatant was evaporated to dryness under nitrogen at 37 °C and reconstituted with 200 μL of 50:50 methanol/water.

Reconstituted samples were quantified using an API 4000 triple quadrupole mass spectrometer equipped with TurboIonSpray™ interface (Applied Biosystems/MDS SCIEX, Foster City, CA, USA) with peak area integration conducted using Analyst Software v 1.4 (Applied BioSystems/MDS SCIEX) data acquisition system. High-performance liquid chromatography separation was achieved using a Phenomenex Luna C18 (Phenomenex®; Torrance, CA, USA) column (30 × 2 mm, 3 μm) with a guard column and a flow rate of 0.3 mL/min. Mobile phase A consisted of 0.5% formic acid in water and mobile phase B consisted of 0.5% formic acid in methanol. The mobile phase gradient started at 30% mobile phase B, switched to 65% mobile phase B from 0 to 0.5 min, and returned to 30% mobile phase B from 2.0 to 2.1 min. The injection volume was 2–30 μL depending on the sensitivity of the system used. Mass spectrometer detection was conducted using positive ionization mode and monitoring of the transitions 337 m/z → 188 m/z for fentanyl and 342 m/z → 188 m/z for the IS fentanyl-d5. Both analytes were typically eluted from the column at 2.95 min. The total run time per sample was 6 minutes. Standard curves were determined using linear regression with 1/x weighting and the peak area ratios of fentanyl to the IS. Typical squared correlation coefficient (R^2) values were 0.9979–0.9999. The precision of the assay was ≤ 5.1%, and the accuracy ranged from −1.9% to 9.3%. The lower limit of quantification (LLOQ) of the method was 0.025 ng/mL.

Data analysis

Plasma fentanyl concentrations less than the LLOQ were set equal to zero ng/mL for descriptive statistic calculations. Pharmacokinetic (PK) parameters were calculated for each subject using noncompartmental PK analysis methods. Plasma samples less than the LLOQ were ignored in the noncompartmental PK analysis, except for the time 0 samples which were replaced by zero ng/mL. The linear up-log down trapezoidal rule was used for the area under the plasma concentration–time curve calculation from time 0 to the time of the last sample at or above the LLOQ ($AUC_{0\text{-}LLOQ}$). Log-linear regression was conducted to determine the terminal first-order rate constant to calculate the total AUC from time 0 to infinity ($AUC_{0\text{-}\infty}$) and the terminal half-life ($t_{½}$). The maximum observed plasma concentration (C_{max}) and time of C_{max} occurrence (t_{max}) were determined directly from the data. All PK parameter calculations were conducted using Phoenix WinNonlin® version 6.1 (Pharsight Corporation, Mountain View, CA, USA).

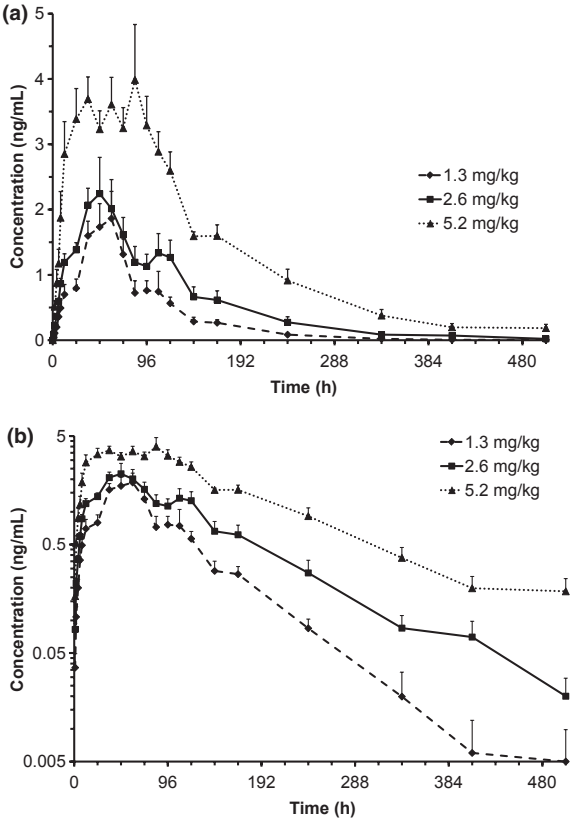

Fig. 1. Mean plasma fentanyl concentrations vs. time by treatment group on a linear (Panel a) and logarithmic (Panel b) scale. Bars represent the standard error.

RESULTS

Following a single topical dose, fentanyl was absorbed from the ventral abdominal application site within hours of application through 504 hours (21 days) in a dose-dependent manner (Fig. 1, Table 1). Fentanyl was rapidly absorbed following application with a mean absorption lag time (t_{lag}) of 0.333 h in the 1.3 mg/kg group and 0 h in the other two treatment groups (Table 2). The mean C_{max} increased with dose and were 2.28, 2.67, and 4.71 ng/mL in the 1.3, 2.6, and 5.2 mg/kg dose groups, respectively. The mean time that C_{max} was achieved (t_{max}) in all groups ranged from approximately 50 to 60 h. Mean terminal half-lives were 53.7, 69.6, and 103 h in the 1.3, 2.6, and 5.2 mg/kg dose groups, respectively. The mean $AUC_{0\text{-}LLOQ}$ from lowest to highest dose groups were 157, 268, and 645 ng·h/mL were dose proportional with a R^2 of 0.9818 (Fig. 2, Table 2).

There were no adverse reactions or application site skin reactions with the exception of one dog in the 5.2 mg/kg dose

Table 1. Mean and standard error (SE) of plasma fentanyl concentrations following application of 1.3, 2.6, or 5.2 mg/kg of transdermal fentanyl solution as a single dose to the ventral abdomen to dogs

	Fentanyl dose mg/kg (μL/kg)					
	1.3 (25)		2.6 (50)		5.2 (100)	
Time (h)	Mean (ng/mL)	SE	Mean (ng/mL)	SE	Mean (ng/mL)	SE
0	0.000	0.000	0.000	0.000	0.000	0.000
1	0.037	0.013	0.083	0.011	0.167	0.044
2	0.108	0.025	0.221	0.024	0.513	0.090
4	0.199	0.041	0.364	0.032	0.876	0.208
6	0.359	0.111	0.592	0.052	1.173	0.219
8	0.492	0.125	0.864	0.082	1.875	0.401
12	0.699	0.154	1.192	0.131	2.851	0.496
24	0.796	0.141	1.383	0.111	3.387	0.466
36	1.598	0.224	2.069	0.258	3.692	0.338
48	1.733	0.358	2.244	0.556	3.230	0.282
60	1.864	0.415	2.013	0.446	3.608	0.416
72	1.314	0.237	1.613	0.267	3.246	0.315
84	0.724	0.186	1.194	0.242	3.985	0.849
96	0.761	0.149	1.131	0.186	3.288	0.445
108	0.744	0.310	1.336	0.297	2.886	0.306
120	0.565	0.095	1.268	0.264	2.594	0.289
144	0.286	0.066	0.663	0.153	1.592	0.066
168	0.267	0.047	0.615	0.140	1.597	0.169
240	0.085	0.018	0.274	0.085	0.914	0.174
336	0.020	0.014	0.085	0.026	0.377	0.093
408	0.006	0.006	0.070	0.028	0.198	0.056
504	0.005	0.005	0.020	0.009	0.185	0.057

Table 2. Pharmacokinetic parameters by treatment group. Values displayed as mean (standard error of the mean)

	Dose		
Parameter	1.3 mg/kg (25 μL/kg)	2.6 mg/kg (50 μL/kg)	5.2 mg/kg (100 μL/kg)
C_{max} (ng/mL)	2.28 (0.362)	2.67 (0.482)	4.71 (0.722)
t_{max} (h)	58.0 (10.9)	52.0 (11.5)	62.0 (7.85)
t_{lag} (h)	0.333 (0.211)	0.0 (0.0)	0.0 (0.0)
$AUC_{0\text{-}LLOQ}$ (ng·h/mL)	157 (16.1)	268 (40.2)	645 (44.5)
$AUC_{0\text{-}\infty}$ (ng·h/mL)	160 (15.7)	275 (38.5)	675 (47.6)
$AUC_{0\text{-}\infty}$ extrapolated (%)	2.75 (0.887)	3.48 (1.74)	4.22 (1.46)
$t½$ (h)	53.7 (8.48)	69.6 (9.66)	103 (10.6)

LLOQ, lower limit of quantification.

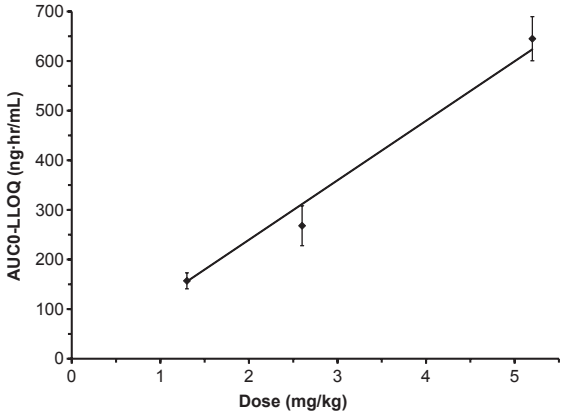

Fig. 2. Mean (±standard error) $AUC_{0\text{-}LLOQ}$ by fentanyl dose. Line represents linear regression with intercept set to zero ($R^2 = 0.9818$).

group that required force feeding and parenteral fluid supplementation owing to the lack of eating and drinking beginning 72 h following dosing. Following intervention for 24 h, the dog appeared normal at 96 h. In addition, four dogs in the 5.2 mg/kg group appeared sedated 8, 24, and 48 h following dosing as well as one dog in the 1.3 mg/kg dose group at 48 h.

DISCUSSION

The results from this study demonstrate that fentanyl can be successfully delivered transdermally with the aid of OS as a penetration enhancer. In all three treatment groups, fentanyl was rapidly absorbed following application to skin and continued for days thereafter in a dose-dependent manner. The certainty that fentanyl was delivered transdermally was confirmed by placing collars on dogs that prevented licking of the ventral abdominal application site. The application site for TFS was later changed during product development to the dorsal interscapular region, eliminating the possibility of licking. Application of TFS to the dorsal interscapular region was differentiated by a more rapid initial absorption of fentanyl compared to the ventral abdominal area (Freise et al., 2012).

Besides the penetration enhancer, an important aspect of the formulation was use of the volatile solvent, isopropanol (Morgan *et al.*, 1998c). Once applied to the skin, rapid solvent evaporation results in super-saturation of both OS and fentanyl. At the moment of drying (approximately 2–5 min following application), rapid dermal absorption and sequestration of fentanyl into the stratum corneum occur. From the stratum corneum, fentanyl is absorbed into the bloodstream over time. As a result of this mechanism, fentanyl undergoes absorption-dependent ('flip-flop') kinetics, that is, the absorption is much longer than the elimination. Therefore, in the plasma concentration–time curve, the downward portion of the curve is the result of decreased drug absorption rather than drug elimination mechanisms. For short half-life drugs such as fentanyl, this process results in an elongated terminal half-life that is because of prolonged absorption. This was confirmed in the present study by the observation of prolonged fentanyl terminal half-lives; all three doses resulted in half-lives ranging from approximately 50 to 100 h. This is in marked contrast to the fentanyl half-life following an intravenous injection of fentanyl citrate where the half-life ranges from 0.76 to 6.0 h (Murphy *et al.*, 1983; Kyles *et al.*, 1996; Hughes & Nolan, 1999; Sano *et al.*, 2006).

The results from this study also portend a potential dose for confirmation in well-controlled laboratory safety studies and clinical trials. The rationale for dose selection is based on the onset and duration of the appearance of the minimum effective plasma concentration (MEC) of fentanyl in each dose group. The MEC is defined as the minimum plasma concentration of an analgesic that is sufficient to prevent a patient from requesting a supplementary analgesic. The MEC of fentanyl has been established to range from 0.2 to 1.2 ng/mL (mean 0.6 ng/mL) in adult, human beings undergoing abdominal surgery (Gourlay *et al.*, 1988). This suggests that the pharmacodynamic variability of minimally effective fentanyl concentrations ranges 6-fold, depending on the individual subject responsiveness.

Establishing the MEC of fentanyl in dogs lacks the sensitivity of the techniques used in human beings. Quantifying the true MEC in dogs remains difficult and depends on a human observer making inferences from presumed pain-related behaviors displayed by dogs. Despite these limitations, behavior-based studies have evaluated analgesia and plasma fentanyl concentration in dogs to approximate analgesia and drug concentrations. The results support the notion that the MEC in dogs likely overlaps with that observed in human beings. Studies in dogs undergoing various surgeries have shown that fentanyl concentrations ranging from 0.4 to 1.28 ng/mL were effective in controlling pain (Kyles *et al.*, 1998; Robinson *et al.*, 1999; Gilbert *et al.*, 2003; Egger *et al.*, 2007). A review and analysis of all studies conducted with fentanyl patches in dogs suggests that a mean plasma fentanyl concentration of 0.6 ng/mL is effective at providing analgesia (Hofmeister & Egger, 2004).

Using a presumed MEC of 0.2–1.2 ng/mL, a dose for further development is proposed. Mean plasma fentanyl concentrations for the 1.3 mg/kg dose group remained at or above the lower and higher end of the MEC range from 4 to 168 h and 36–72 h, respectively (Table 1). In contrast, at the 2.6 mg/kg dose, the mean plasma fentanyl concentrations remained ≥0.2 ng/mL from 2 to 240 h and ≥1.2 ng/mL from 12 to approximately 84–120 h. With the higher dose of 5.2 mg/kg, mean concentrations remained ≥0.2 ng/mL from 1 to 504 h and ≥1.2 ng/mL from 6 to 168 h. Thus the onset of a 1.3 mg/kg dose could range from 4 to 36 h with a duration of action of 3–7 days. For a dose of 2.6 mg/kg, the onset could range from 2 to 12 h with a duration of 3.5–10 days. Finally, for a dose of 5.2 mg/kg, the onset could range from 1 to 8 h with a duration of 7–17 days.

To establish a dose, both safety and effectiveness must be considered. In human beings, a dose-limiting effect is opioid-induced hypercapnia and respiratory depression. Such a profound response in humans has resulted in the contraindication fentanyl patches for postoperative pain. In contrast, spontaneous respirations are maintained in dogs independent of fentanyl concentration (Bailey *et al.*, 1987; Mathews, 2000). Plasma fentanyl concentrations as high as approximately 80 ng/mL are not fatal and reduce the respiratory rate by only approximately 11 breaths/minute (50%) in spontaneously breathing dogs (Arndt *et al.*, 1984). Additionally, the respiratory rate, oxygen consumption, and blood gases (pCO_2, pO_2, and pH) do not change further as concentrations increase above 100 ng/mL. The mean C_{max} from the lowest to the highest dose in the present study were 2.28, 2.67, and 4.71 ng/mL, well below the concentrations that have a clinical impact on respiratory rates in dogs. The primary dose-limiting effect appeared to be decreased appetite and sedation in the 5.2 mg/kg dose group. One dog in this dose group required parenteral fluid therapy and forced feeding from 72 to 96 h owing to the lack of food and water intake, and four dogs were sedated for 3 days. The 2.6 mg/kg group did not have any adverse events and had a faster presumed onset of action and longer duration than the 1.3 mg/kg group. Therefore, a dose of 2.6 mg/kg is proposed as a dose for further study in safety and effectiveness trials.

The results from this study demonstrate that the dermal barrier to drug permeation can be overcome for fentanyl using a penetration enhancer and rapidly evaporating solvent. Through deposition of fentanyl in the stratum corneum followed by prolonged systemic absorption, TFS overcomes the limitations of poor oral bioavailability and short duration of action of orally and parenterally administered fentanyl. Moreover, TFS provides several advantages over patches that include: 1.) a rapid time to achieve minimally effective plasma concentrations, 2.) long duration action, 3.) no requirement for maintenance of device contact with skin to maintain fentanyl absorption, 4.) dosing on a per kilogram basis that results in dose proportional pharmacokinetics therefore overcoming problems with variable rate and extent of patch delivered fentanyl, 5.) elimination of inadvertent patch exposure to the dog or dog owner, and 6.) avoidance of diversion and illicit use of patches outside the control of a licensed veterinarian. Futhermore, as an approved product in dogs, required pharmacovigilance and adverse events tracking will aid veterinarians making better informed decisions regarding its use compared to the off-label use of the human approved fentanyl products.

CONCLUSION

In summary, the pharmacokinetics of 1.3 (25), 2.6 (50), and 5.2 mg/kg (100 μL/kg) of TFS was examined in 18 healthy, laboratory Beagles (three males and three females per group). The PKs were characterized by a rapid initial absorption of fentanyl, within hours of application, followed by a slow terminal decline in the plasma fentanyl over a period of days controlled by 'flip-flop' kinetics. Maximum plasma concentrations from lowest to highest dose were 2.28, 2.67, and 4.71 ng/mL. The PKs were dose proportional and half-lives ranged from approximately 50 to 100 h. A dose of 2.6 mg/kg is proposed based on the lack of adverse events observed that were observed in the 5.2 mg/kg group and a more rapid onset of action and longer duration of action compared to the 1.3 mg/kg group.

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CONFLICTS OF INTEREST

The authors were employees or paid contributors to Nexcyon Pharmaceuticals, Inc.

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