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Pharmacokinetics and the effect of application site on a novel, long-acting transdermal fentanyl solution in healthy laboratory Beagles

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Application of transdermal drugs to different anatomical sites can result in different absorption characteristics. The pharmacokinetics (PKs) and bioequivalence of a single 2.6 mg/kg (50 μL/kg) dose of a novel, long-acting transdermal fentanyl solution were determined when applied topically to the ventral abdominal or dorsal interscapular skin of 40 healthy laboratory Beagles. The PKs were differentiated by a more rapid initial absorption of fentanyl from the dorsal application site. Mean plasma fentanyl concentrations remained above 0.6 ng/mL from 4 to 96 h in the dorsal application group and from 8 to 144 h in the ventral application group. Bioequivalence analysis demonstrated that the sites were not equivalent; the 90% confidence intervals of the ratio of the geometric means for both the maximum concentration (C_{max}) and the area under the curve (AUC) were not contained within the 80–125% interval. The C_{max} was 2.34 ± 1.29 (mean ± standard deviation) and 2.02 ± 0.84 ng/mL for the ventral and dorsal application groups, respectively. The terminal elimination half-lives ($t_{1/2}$) for both groups were similar with values of 137 ± 58.9 and 117 ± 59.6 h for the ventral and dorsal application site groups, respectively. A mean absorption rate of ≥2 μg · kg/h was maintained from 2 to 144 h following dorsal application and from 2 to 264 h following ventral application. These results suggest that transdermal fentanyl solution could be applied as a single dose to the dorsal scapular area 2–4 h prior to surgery with analgesia lasting a minimum of 4 days.

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INTRODUCTION

Poor bioavailability and rapid clearance of oral and parenteral opioids in dogs have limited these pharmaceutical forms to single or repeat perioperative parenteral injections or constant rate intravenous infusions (CRI) delivered during anesthesia (Pascoe, 2000). As a pharmaceutical delivery strategy to extend the duration of action, transdermal application has several potential advantages over oral or parenteral administration that include noninvasive dosing, avoidance of the gastrointestinal tract, lack of first-pass metabolism, steady, continuous drug delivery rather than a peak and trough phenomenon, potential reduction in side effects by the elimination of peaks, possible reduction in end-of-dose lack of effectiveness owing to elimination of troughs and reduced dose frequency for convenience and increased compliance.

A previous study demonstrated that a novel, long-acting transdermal fentanyl solution resulted in dose-dependent plasma fentanyl concentrations (Freise *et al.*, 2012). A dose of 2.6 mg/kg (50 μL/kg) was proposed as a single topical application to the ventral abdomen that provided potential analgesic onset of action within hours and an extended duration for days. There may be advantages of applying the solution to sites other than the ventral abdomen. For example, dorsal interscapular application allows ambulatory dogs to be walked to and from a treatment area for ease of application and is far from a laparotomy surgical site. However, different transdermal application sites are known to result in dissimilar drug delivery characteristics. For example, in humans, sites with greatest to least potential for drug absorption are scrotal > forehead > axilla/scalp > back/abdomen > palmar and plantar (Feldmann & Maibach, 1967). The abdomen vs. back skin in

dogs has been shown to differ with regard to blood flow, and therefore different absorption characteristics may be apparent when drugs are applied to these sites (Monteiro-Riviere *et al.*, 1990). Therefore, the objective of this study was to examine the pharmacokinetics (PKs) of transdermal fentanyl solution when applied topically to the ventral abdominal vs. the dorsal interscapular areas.

MATERIALS AND METHODS

Pilot intravenous study

In order to calculate the fentanyl unit impulse response to determine the transdermal fentanyl absorption rate using deconvolution, a pilot study of intravenously administered fentanyl citrate was conducted (Veng-Pedersen, 2001). Three healthy purpose-bred laboratory Beagles (two males/one female) weighing 10.7 ± 1.1 (mean ± standard deviation) kg were randomly selected for a pilot study of intravenous (IV) fentanyl citrate administration. Dogs were individually housed, fed a commercial dry food formula, and allowed *ad libitum* access to water. All procedures were approved by the local Institutional Animal Care and Use Committee.

Fentanyl citrate (Sigma-Aldrich, St. Louis, MO, USA) for IV administration was dissolved in 0.9% sodium chloride at a free base concentration of 35 μg/mL, pH adjusted to 6.5–7.5 with sodium hydroxide and sterile filtered through a 0.2-μm filter on the day prior to dosing. Dogs were fasted overnight prior to dosing. Each dog was administered a 6.6 μg/kg IV bolus dose of fentanyl citrate through a cephalic vein catheter. Following injection, the catheters were flushed with 3 mL of sterile 10 IU/mL heparinized saline and removed. Two millilitres of blood samples was collected via jugular venipuncture into heparinized glass tubes at time 0 (prior to dosing) and at 5, 15, 30, 45 min and 1.25, 2, 4, 8, and 12 h postdosing. Each sample was immediately placed on ice and centrifuged at 2000 ***g*** for 15 min at 4 °C. Plasma was pipetted into plastic sample collection tubes and frozen at −20 °C until assayed.

Transdermal fentanyl solution study

The investigational drug was TFS (Recuvyra™ transdermal solution [fentanyl]; Nexcyon Pharmaceuticals Ltd, London, UK). It is a clear, colorless to light yellow solution that contains 5% w/v (50 mg/mL) fentanyl base, the skin penetration enhancer octyl salicylate (also known as octisalate and 2-ethylhexyl salicylate) at a concentration of 5% w/v (50 mg/mL) in isopropanol (qs).

Forty purpose-bred laboratory Beagle dogs (Marshall BioResources, North Rose, NY, USA) were selected; there were 20 males and 20 females, all 6–8 months of age at the time of dosing. Females were nulliparous and nonpregnant. The weight range for the animals at the time of allocation to groups was 5.55–8.40 kg for males and 4.25–7.35 kg for females. Dogs were kept indoors, one dog per cage. Day 0 was defined as the day of dosing, and dogs were allowed 5 days of acclimation prior to day 0. All animals were acclimated to the collars (Bite-Not® 4–5 inches; Bite Not Products, Inc., San Francisco, CA, USA) 2 days prior to dosing for approximately 2 h to prevent direct licking of the ventral application site. One day prior to dosing, all dogs allotted to ventral dosing were acclimated to the collars for approximately 4 additional hours. All procedures were approved by the local Institutional Animal Care and Use Committee.

The most recent body weight (day 1) was used to calculate each dose. Dogs were administered 2.6 mg/kg (50 μL/kg) of transdermal fentanyl solution applied as a single dose to the dorsal interscapular region or ventral abdominal skin near the umbilicus based on the previously selected dose (Freise *et al.*, 2012). Within sex, dogs were randomly allotted to dorsal or ventral dosing such that each group had 10 males and 10 females (20 per group) in a parallel study design. The animals were fed before treatment on day 0 consistent with the feeding schedule during acclimation. The test article was applied directly onto the skin in the desired area of application, using a proprietary applicator tip and syringe (patent pending). The applicator tip was designed with a fork-like bifurcation (Fig. 1) to channel the liquid into two distinct spots when applied to the skin. The syringe was siliconized and contained a silicone O-ring to accommodate the isopropyl alcohol content of the investigational formulation. Prior to topical application, there was no preparation of the dorsal or ventral application sites other than confirming that the site was free of gross debris and the skin was intact and undamaged. With the calculated volume collected into the syringe, the applicator tip was attached via a luer-lock mechanism. The applicator tip was placed at an approximate 45° angle directly onto the skin over the dorsal or ventral application site area. Without moving the applicator tip, the calculated volume was applied to the skin. Dose volumes ranged from 0.21 to 0.42 mL. Dogs were restrained for a full 2 min to allow the liquid to evaporate, and contact with the application site was avoided for 5 min. Collars were placed on animals that received

Fig. 1. The applicator tip used to apply transdermal fentanyl solution to the ventral abdominal and dorsal interscapular skin.

the dose on the ventral abdomen to prevent access to the treated site and were left on the animals for approximately 48 h.

Serial venous blood samples were collected at 0 (predosing), 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 216, 264, 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). For the plasma samples collected from the transdermal fentanyl solution portion of the study, a 1 mg/mL stock solution of fentanyl (Cerilliant®, Round Rock, TX, USA) dissolved in methanol was diluted in 50:50 methanol (Honeywell Burdick & Jackson®, Morristown, NJ, USA)/water (Milli-Q; Millipore Corp., Billerica, MA, USA) to a 25 μg/mL working solution. Control dog plasma (Bioreclamation Inc., Hicksville, NY, USA) was then serially diluted with the fentanyl working solution to create standard curve samples ranging from 0.1 to 100 ng/mL (10 calibration standards in duplicate) and quality control (QC) samples at concentrations of 0.1, 0.3, 3.5, 40, and 85 ng/mL. Additionally, 100 μg/mL stock solution of the internal standard (IS) fentanyl-d_5 (Cerilliant®) dissolved in methanol was diluted in 50:50 methanol/water to a 200 ng/mL working solution. One hundred microliters each of sample, standard, QC, or control blank was aliquoted directly into a 96-well block, and 20 μL of the IS working solution was added to all wells except for the control blanks. Twenty microliters of 50:50 methanol/water were added to the control blanks instead of the IS working solution, and all samples were vortexed for 30 s. Four hundred microliters of 5% acetic acid (Mallinckrodt Baker, Phillipsburg, NJ, USA) in water was then added to each well, and the samples were vortexed again followed by centrifugation at 2000 ***g*** and 4 °C. Solid-phase extraction (SPE) was then proceeded using Bond Elut® 96 Certify, 50 mg sample extraction blocks (Varian Corp., Palo Alto, CA, USA), and a Tomtec Quadra-96 Model 320 (Tomtech, Hamden, CT, USA). Sample blocks were preconditioned with 1 mL methanol followed by 1 mL water. Samples were then transferred to the extraction blocks and gently pulled through the SPE plate with low vacuum, followed by a wash with 1 mL of 5% acetic acid in water and then by 1 mL of methanol. Samples were eluted through the extraction blocks with 600 μL of 2% ammonium hydroxide (EMD Biosciences, Darmstadt, Germany) in acetonitrile (Honeywell Burdick & Jackson®), into a new collection plate. Eluted samples were evaporated under nitrogen at 40–45 °C to dryness with about 35 F^3/h flow and reconstituted with 200 μL of 1% formic acid (EMD Biosciences) in acetonitrile.

Reconstituted samples were quantified using an API 3000 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 LC-10AD pumps (Shimadzu Co., Kyoto, Japan) and a Thermo Betasil Silica-100 column (50 × 3 mm, 5 μm) (Thermo Fisher Scientific, Waltham, MA, USA) with the flow rate set at 0.5 mL/min. Mobile phase A consisted of 1% formic acid in water and mobile phase B consisted of 1% formic acid in acetonitrile. The mobile phase gradient started at 90% mobile phase B from 0.0 to 1.0 min, switched from 90 to 70% mobile phase B from 1.0 to 1.5 min, and switched back from 70 to 90% mobile phase B at 2.5 min. The total run time per sample was 2.7 min. The injection volume was 1 μL, and mass spectrometer detection was conducted using positive ionization mode and monitoring of the transitions 337.2 m/z → 188.3 m/z for fentanyl and 342.2 m/z → 188.3 m/z for the IS fentanyl-d_5. Both analytes were typically retained on the column for 1.93 min. Standard curves were determined using linear regression with $1/x^2$ weighting using Watson v7.0.0.01 (Thermo Fisher Scientific), where x is the nominal sample concentration and had typical squared correlation coefficient (R^2) values of 0.9987 (range of 0.9968–0.9996). All concentration calculations were based on the peak area ratios of fentanyl to the IS. The intra- and interassay precision was ≤ 7.3%, and the accuracy ranged from −13.5 to 5.9%. The lower limit of quantification (LLOQ) of the method was 0.100 ng/mL.

Data analysis

Plasma fentanyl concentration <LLOQ was set equal to 0 ng/mL for descriptive statistic calculations. PK parameters from the transdermal fentanyl solution study were calculated using noncompartmental PK analysis methods. 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 ($AUC_{0–LLOQ}$). Log-linear regression was conducted to determine the terminal elimination first-order rate constant (λ_Z). The number of data points to include in the λ_Z calculation was determined by maximizing an adjusted R^2 function from the last three data points above the LLOQ to the last n data points from the maximum observed plasma concentration (C_{max}), as detailed in the Phoeneix WinNonlin® Version 6.1 documentation (Pharsight Corporation, Mountain View, CA, USA).

A bioequivalence analysis between the two dosing sites was conducted on the log-transformed C_{max} and $AUC_{0–LLOQ}$ PK parameters with group as the independent variable. Ventral application was considered the reference treatment and the dorsal application the test treatment. Ninety percent confidence intervals (CI) of the ratio of dorsal to ventral geometric means were constructed, and bioequivalence was determined as described in the Bioequivalence Guidance (FDA-CVM, 2002). The two application sites were considered bioequivalent if the 90% CI of the ratio of the geometric means was completely contained within the 80–125% interval. All PK parameter calculations and parameter analyses were conducted using Phoenix WinNonlin® Version 6.1.

A two-compartment model parameterized as macro-parameters (i.e., a polyexponential function) was fit to the IV fentanyl

plasma concentration–time data. The average macro-parameters were used as the population unit impulse response to calculate the mean systemic absorption rates and bioavailability using deconvolution for ventrally and dorsally applied transdermal fentanyl solution. The deconvolution analysis was conducted using the Deconvolution Tool in Phoenix WinNonlin® Version 6.1.

Quality assurance

The transdermal fentanyl solution study was conducted and reported in compliance with the United States Food and Drug Administration (FDA), Department of Health and Human Services, Good Laboratory Practices (Title 21 Code of Federal Regulations Part 58).

RESULTS

There were no adverse events in this study. A single dog in the dorsal application group had a plasma fentanyl concentration of 13.0 ng/mL at 96 h compared to the group mean of 0.61 ng/mL. Moderate to severe sedation would be expected at concentrations near or above 15 ng/mL (Savides *et al.*, 2012). No sedation or adverse events were noted at the time of the observed elevated concentration at 96 h, and the 72- and 120-h plasma fentanyl concentrations in this subject were below 1.0 ng/mL. Therefore, the transient spike was considered spurious and was dropped from further PK analysis.

The mean plasma fentanyl concentrations by application site are displayed in Table 1 and Fig. 2. Plasma fentanyl concentra-

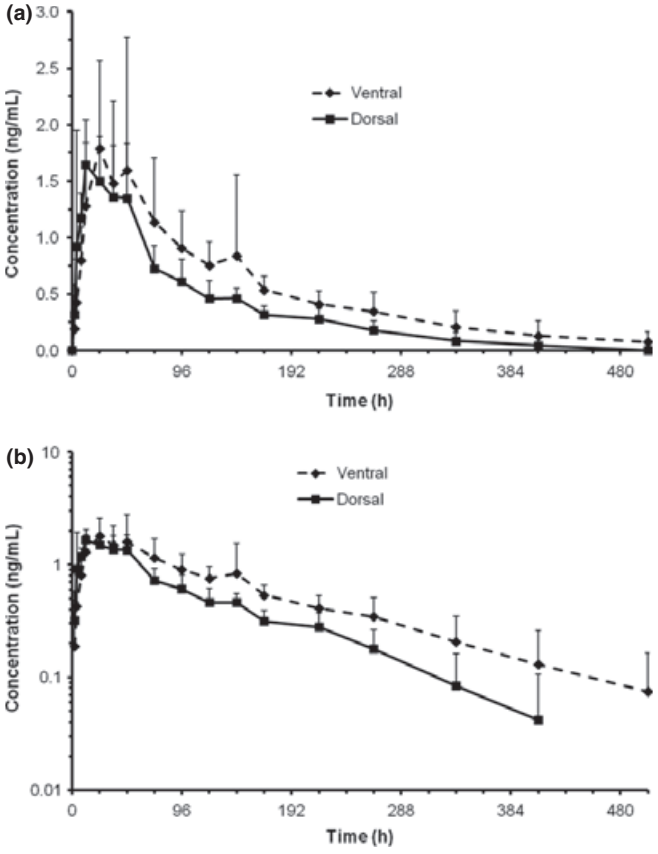

Fig. 2. Mean plasma fentanyl concentrations following transdermal fentanyl solution administration on a linear (a) and logarithmic (b) scale. Bars represent the standard deviation.

Table 1. Transdermal fentanyl solution summary statistics* of plasma fentanyl concentrations by treatment group (n = 20/group)

	Ventral application			Dorsal application		
Time (h)	Mean (ng/mL)	Standard deviation (ng/mL)	C.V. (%)	Mean (ng/mL)	Standard deviation (ng/mL)	C.V. (%)
0	0.000	0.000	NA	0.000	0.000	NA
2	0.189	0.298	158.1	0.319	0.265	83.1
4	0.425	0.384	90.3	0.920	1.028	111.7
8	0.796	0.294	36.9	1.18	0.215	18.3
12	1.28	0.557	43.5	1.65	0.391	23.7
24	1.79	0.775	43.3	1.50	0.401	26.8
36	1.48	0.728	49.1	1.36	0.453	33.3
48	1.59	1.18	73.9	1.35	0.483	35.8
72	1.14	0.571	50.2	0.725	0.200	27.6
96	0.907	0.332	36.6	0.610	0.200	33.1
120	0.755	0.204	27.1	0.458	0.157	34.4
144	0.835	0.714	85.5	0.461	0.0887	19.2
168	0.536	0.123	22.9	0.315	0.0790	25.1
216	0.411	0.117	28.5	0.278	0.0927	33.4
264	0.343	0.169	49.3	0.178	0.0848	47.6
336	0.206	0.142	68.7	0.0842	0.0770	91.4
408	0.129	0.133	102.6	0.0418	0.0646	154.5
504	0.074	0.090	120.6	0.000	0.000	NA

C.V.: Coefficient of variation; NA: Not applicable. *Concentrations <LLOQ (0.100 ng/mL) were set equal to zero.

tions rose more rapidly following dorsal application and persisted longer in the ventral application group. Mean plasma fentanyl concentrations remained above 0.6 ng/mL, and the mean concentration considered to be analgesic (Hofmeister & Egger, 2004), from 4 to 96 h in the dorsal application group and 8–144 h in the ventral application group.

The PK parameters by application site group are summarized in Table 2. The C_{max} was 2.34 ± 1.29 (mean ± standard deviation) and 2.02 ± 0.84 ng/mL for the ventral and dorsal application groups, respectively. The t_{max} was 40.2 ± 29.5 and 24.8 ± 17.8 h in the ventral and dorsal application site groups, respectively. The terminal elimination half-lives ($t_{1/2}$) for both groups were similar with values of 137 ± 58.9 and 117 ± 59.6 h for the ventral and dorsal application site groups, respectively. Less that 20% of the $AUC_{0-\infty}$ was extrapolated for both groups, indicating that AUC_{0-LLOQ} sufficiently reflects the extent of exposure.

The bioequivalence analysis results are displayed in Table 3. The dorsal to ventral ratio of the geometric means for AUC_{0-LLOQ} was 70.5% (90% CI [60.6–82.0%]), and for C_{max}, ratio of the geometric means was 93.1% (90% CI [73.4–118%]).

The PK parameters calculated from the IV administration of fentanyl citrate in the pilot study are summarized in Table 4. The mean terminal (β) half-life of IV administered fentanyl was relatively short at only 2.66 h, and the mean volume of distribution at steady-state (V_{SS}) was relatively large at 5.09 L/kg. The calculated absorption rates and cumulative absorption for ventrally and dorsally administered transdermal fentanyl solution using deconvolution are depicted in Fig. 3. The cumulative absorption amounts (and thus the average absorption rates) were very similar in both application site groups until about 72 h, when the ventral application site absorption started to become greater than that following dorsal application. At 504 h, the bioavailability for ventral and dorsal application was 49.1% and 34.2%, respectively.

Table 2. Transdermal fentanyl solution pharmacokinetic parameters by treatment group (n = 20/group)

	Ventral application		Dorsal application	
Parameters	Mean	Standard deviation	Mean	Standard deviation
C_{max} (ng/mL)	2.34	1.29	2.02	0.840
t_{max} (h)	40.2	29.5	24.8	17.8
AUC_{0-LLOQ} (ng · h/mL)	251	75.1	170	29.0
$AUC_{0-\infty}$ (ng · h/mL)	282	82.5	198	33.9
$AUC_{0-\infty}$ extrapolated (%)	11.6	5.06	13.4	7.40
$t_{½}$ (h)	137	58.9	117	59.6

Table 3. Ratio (dorsal/ventral) of the geometric means and 90% confidence interval of select transdermal fentanyl solution pharmacokinetic parameters (n = 20/group)

Parameters	Lower 90% confidence interval	Ration of the geometric means	Upper 90% confidence interval
AUC_{0-LLOQ} (%)	60.6	70.5	82.0
C_{max} (%)	73.4	93.1	118

DISCUSSION

This study demonstrates that the absorption of transdermal fentanyl solution is not equivalent when applied to the dorsal

Table 4. Unit impulse response and pharmacokinetic parameters for a single IV injection of fentanyl citrate (6.6 μg/kg) (n = 3)

Parameters	Mean	Standard deviation
A (kg/mL)	1.92×10^{-4}	6.33×10^{-5}
B (kg/mL)	3.43×10^{-5}	1.67×10^{-5}
α (1/h)	2.594	1.13
β (1/h)	0.268	0.0601
α half-life (h)	0.298	0.106
β half-life (h)	2.66	0.520
Cl (mL · h/kg)	1.32	0.187
V_{SS} (L/kg)	5.09	0.768

A, B: macro-parameter constants; α, β: macro-parameter rate constants; Cl: clearance; V_{SS}: apparent volume of distribution at steady-state.

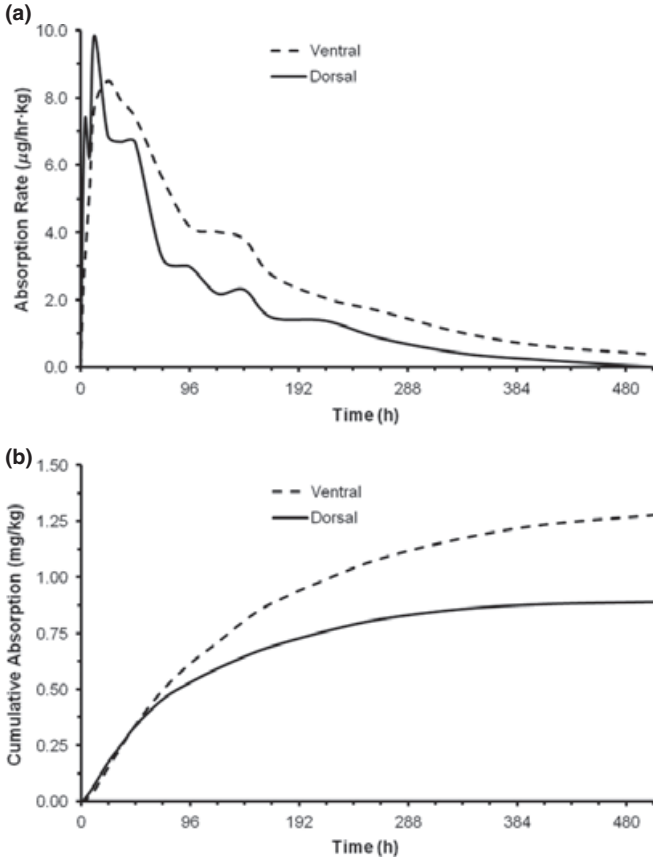

Fig. 3. Mean systemic fentanyl absorption following transdermal fentanyl solution administration. Panel (a) displays the absorption rate and Panel (b) the cumulative amount absorbed.

interscapular area vs. the ventral abdominal area. Absorption was more rapid for dorsally applied transdermal fentanyl solution (Figs 2 and 3), supporting a more rapid onset of action compared to ventral application. While the minimal effective concentration (MEC) in dogs has not been well defined, studies in humans and dogs have supported the notion that fentanyl concentrations from 0.2 to 1.2 are minimally effective for the control of postoperative pain (Gourlay *et al.*, 1988; Hofmeister & Egger, 2004). Mean plasma fentanyl concentrations of 0.6 ng/mL have been considered to be analgesic in dogs (Gourlay *et al.*, 1988; Hofmeister & Egger, 2004). Whereas the mean time to achieve a concentration of 0.6 ng/mL was 4 h for dorsal application, ventral application did reach this concentration until 8 h following dosing. The more rapid absorption associated with dorsal application was also associated with a difference in mean duration above 0.6 ng/mL. The mean time to drop below 0.6 ng/mL for dorsal and ventral application was 96 and 144 h, respectively (Table 1). The relatively large coefficients of variation (C.V.) in plasma fentanyl concentrations do mean there may be significant subject-to-subject variability in both the onset and duration of action of transdermal fentanyl solution applied to either site. However, the variability is not substantially more than that achieved with the off-label use of the transdermal fentanyl patch in dogs (Kyles *et al.*, 1996), particularly after dorsal application.

The lack of similarity between application sites was confirmed by a bioequivalence analysis. Based on a 90% CI of 80–125% for bioequivalence determination, the C_{max} and AUC_{0-LLOQ} were not equivalent (Table 3). Furthermore, as the 90% CI for AUC_{0-LLOQ} does not contain 100%, there is confirmatory evidence that systemic exposure (i.e., the extent of absorption) following dorsal application is less than that following ventral application. Both application sites were characterized by a long terminal phase with a $t_{1/2}$ exceeding 100 h (Table 2), similar to that previously reported (Freise *et al.*, 2012), indicating flip-flop PKs.

The pilot study with IV fentanyl citrate resulted in PK parameters consistent with other reports (Murphy *et al.*, 1983; Kyles *et al.*, 1996; Sano *et al.*, 2006). These data were used to calculate the absorption rates and bioavailability from the two application sites examined in this study. The recommended infusion rate targets when fentanyl is administered by CRI are 2–10 $\mu g \cdot kg/h$ (Plumb, 2008). Dorsal administration achieved an absorption rate of $\geq 2\ \mu g \cdot kg/h$ from 2 to 144 h following application with a peak of 9.8 $\mu g \cdot kg/h$ occurring at 12 h (Fig. 3). In contrast, ventral administration achieved an absorption rate of $\geq 2\ \mu g \cdot kg/h$ from 2 to 264 h with a peak of 8.5 $\mu g \cdot kg/h$ occurring at 24 h. These results suggest that potentially analgesic infusion rates from transdermal fentanyl solution are achieved within a few hours of application and are maintained for periods of up to 10 days.

The bioavailability at 504 h for ventral and dorsal was 49.1% and 34.2%, respectively, with possible continued absorption beyond 504 h (Fig. 3). The skin provides an effective barrier to prevent passage of xenobiotics and limits drug absorption. The mechanism for the differences in absorption rates and bioavailability observed between the dorsal and ventral application site is beyond the scope of this study; however, there are several possibilities including alteration in the epidermis morphology (Monteiro-Riviere, 1990), skin surface makeup such as oil and sebaceous secretions (Allen & Monteiro-Riviere, 1999), hair follicle density (Smith *et al.*, 1990), rate of solvent evaporation (Magnusson *et al.*, 2001), stratum cornuem thickness (Monteiro-Riviere *et al.*, 1990), altered biotransformation (Mukhtar, 1992), and altered vascular function (Riviere *et al.*, 1992). In dogs, the stratum corneum and epidermal thickness as well as the number of cell layers in the skin of the abdomen and back has been reported to be similar (Monteiro-Riviere *et al.*, 1990). However, blood flow between these two dermal sites is different at 8.78 and 1.94 mL · min/100 g for the abdomen and back, respectively. Therefore, it is possible that the differences in absorption and bioavailability associated with dorsal application may be related to differences in blood flow compared to the ventral abdomen skin.

The results support a dose for further evaluation in laboratory safety studies and well-controlled field studies in client-owned dogs. A previous dose titration study with this formulation supported a dose of 2.6 mg/kg (50 μL/kg) applied as a single dose to the ventral abdominal skin to provide analgesia with an onset of action ranging from 2 to 12 h and duration of action from 3.5 to 10 days (Freise *et al.*, 2012). The rationale for the proposed dose selection was based on the onset and duration of the appearance of the MEC of fentanyl in three dose groups. The present study adds additional information leading to the proposal of a refined dosage regimen for further study. Both application sites resulted in absorption rates of $>2\ \mu g \cdot kg/h$ within 2 h of topical application, but the dorsal site achieved mean plasma concentration of 0.6 ng/mL by 4 h compared to 8 h with ventral application. When taken together, dorsal application provides a more rapid onset of action. With such a rapid onset of action, transdermal fentanyl solution could be applied to a dog as it enters the hospital as an anesthetic premedication with analgesic concentrations potentially occurring 2–4 h prior to surgery. Without additional opioid administration, this single dose could have a duration of a minimum of 4 days. Confirmation of this likely outcome requires well-controlled field studies in client-owned dogs.

CONCLUSION

In summary, the PKs and bioequivalence of a single 2.6 mg/kg (50 μL/kg) dose of transdermal fentanyl solution were determined when applied topically to the ventral abdominal or dorsal interscapular skin in 40 healthy laboratory Beagles. The PKs were differentiated by a more rapid initial absorption of fentanyl following dorsal application. Bioequivalence analysis demonstrated that the sites were not equivalent; the 90% CI for both the C_{max} and the AUC_{0-LLOQ} was not contained within the 80–125% interval. Both application sites were characterized by slow terminal decline in the plasma fentanyl concentrations over time with concentrations dropping below 0.6 ng/mL by 96 and 144 h for dorsal and ventral sites, respectively. An absorption

rate of ≥2 $\mu g \cdot kg/h$ was from 2 to 144 h following dorsal application and 2 to 264 h following ventral application. These results suggest that transdermal fentanyl solution could be applied as a single dose dorsally 2–4 h prior to surgery with analgesia lasting a minimum of 4 days.

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CONFLICTS OF INTEREST

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

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