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In vitro characterization of the percutaneous absorption of tramadol into inner ear domestic feline skin using the Franz skin finite dose model

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Abstract

Pain, whether it is acute or chronic, can induce stress and reduce overall quality of life for domestic felines. Tramadol, a synthetic opioid that exerts analgesic effects by binding to μ-opioid receptors as well as inhibiting neuronal reuptake of norepinephrine and serotonin, can be used alone or in combination with NSAIDs for the treatment of domestic feline pain. Tramadol can be incorporated into transdermal bases such as PLO or Lipoderm for transdermal delivery. The purpose of this study is to characterize the percutaneous absorption of two tramadol formulations (Tramadol 100 mg/g in PLO and Tramadol 100 mg/g in Lipoderm), when applied to the inner ear of domestic feline skin, *in vitro*, using the Franz skin finite dose model. A variable finite dose (e.g., 25 μg/cm^2) of each formulation was applied to skin sections from two donors and cultured within Franz diffusion cells for 48 hr. Tramadol total absorption, rate of absorption, surface wash, and skin content were quantified using HPLC analysis. Results show that both PLO and Lipoderm were capable of facilitating the percutaneous absorption of tramadol across *ex-vivo* domestic feline inner ear skin. However, tramadol content within surface wash (3.65%±0.14) was lower for the Lipoderm formulation, which potentially correlates to the higher total absorption (100.40%±3.24) of tramadol when in Lipoderm. Although the rise to peak rate of absorption was approximately 2.5 hr for both formulations, the decline in rate was steadier and more predictable for tramadol in Lipoderm than in PLO. Results of this study can help practitioners and pharmacists predict the *in vivo* percutaneous absorption of tramadol in domestic felines. The prediction can then guide them in selecting the most favorable transdermal base when prescribing and compounding with tramadol for domestic feline use.

Keywords: Domestic feline(s), pain, pharmaceutical compounding, percutaneous absorption, tramadol, Lipoderm, PLO, Franz skin finite dose model

Introduction

Companion animals such as cats and dogs are popular pets in many countries around the world. In the United States, the estimated percentages of households owning pets are 30.4% for cats and 36.5% for dogs [1]. Though both domestic animals are cherished by their caregivers, cats are often under-treated for pain in comparison to dogs. Domestic cats, also referred to as domestic felines, belong to the larger family of felines, which also includes tigers, lions, jaguars, and wild cats. Only 56% of domestic felines receive adequate analgesia due to the increased difficulty for caregivers to recognize and assess pain in these animals [2].

The types of pain experienced by domestic felines can be divided into two categories: acute pain and chronic pain. Acute pain is often a result of trauma or surgery, while chronic pain is usually associated with Degenerative Joint Disease (DJD), a condition that involves deterioration of tissues surrounding the joints. Opioids such as morphine or fentanyl are commonly used for acute pain, while Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) such as meloxicam or ketoprofen are commonly used for chronic pain [3]. Though opioids and NSAIDs are common treatment options, there are disadvantages with the use of these medications in domestic felines. As obligated carnivores, the feline family has low capacity for the metabolism of drugs via hepatic glucuronidation, a pathway that develops as a result of exposure to phytoalexins in plants. Deficiency in this pathway can lead to decreased efficacy with morphine, as the glucuronidation pathway is required to convert morphine to

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its active metabolite, Morphine-6-Glucuronide (M-6-G) [2,3]. Although NSAIDs do not require the glucuronidation pathway for activation, long-term exposure to this medication class can lead to gastrointestinal toxicity (e.g., bleeding, ulcerations) and renal damage, which limits the chronic use of NSAIDs [3]. Due to the limitations of current therapies, pain management in domestic felines has become increasingly challenging for practitioners and pharmacists.

In recent years, tramadol has been researched for the treatment of domestic feline pain. Tramadol is a synthetic opioid that exerts analgesic effects by binding to μ-opioid receptors as well as inhibiting neuronal reuptake of norepinephrine and serotonin [4]. Previous studies have shown that tramadol can be used alone or in combination with NSAIDs for the treatment of mild to moderate pain in domestic felines [5,6]. The conventional routes of administration for tramadol include oral, Intravenous (IV), and Subcutaneous (SC) administration [5]. Both IV and SC administration are invasive and, therefore, it may be challenging for caregivers to successfully administer tramadol in a home environment. Although oral administration of tramadol is non-invasive, this route of administration requires physical restraint of the animal by its owner to administer each dose, which can lead to non-compliance and treatment failure [3].

The Food and Drug Administration (FDA) has recognized the need for pharmaceutical compounding in veterinary medicine. Particularly with regards to domestic felines, pharmaceutical compounding can be utilized to overcome the limitations of conventional therapies [7]. Pharmaceutical compounding involves the preparation of customized medications to meet the individual patient needs [8]. When compounded, tramadol can be incorporated into transdermal bases such as Pluronic Lecithin Organogel (PLO) or Lipoderm for transdermal delivery. In comparison to oral, IV, and SC administration, transdermal delivery is a non-invasive route of administration that allows for the percutaneous absorption of drugs. Following application, the medication is intended to be directly absorbed into systemic circulation over a period of time, bypassing first-pass metabolism [9].

PLO is a first generation transdermal base that contains Pluronic F-127, soya lecithin, and IPP (Isopropyl Palmitate)/IPM (Isopropyl Myristate). PLO is composed of two phases, an aqueous phase (pluronic phase) and an oil phase (lecithin phase), continuously mixed with one another until a homogenous gel is formed. The gel is intended to dissolve both hydrophobic and hydrophilic drugs, with hydrophobic drugs dissolving in the oil phase and hydrophilic drugs dissolving in the aqueous phase [10]. Lipoderm, on the other hand, is a transdermal base that consists of a proprietary liposomal component shown to successfully facilitate the penetration of drugs into and through *ex vivo* human skin under *in vitro* conditions [11]. Although transdermal delivery of tramadol in PLO or Lipoderm is advantageous in comparison to conventional routes, there is limited data to support the efficacy of compounded tramadol for transdermal delivery in domestic felines. The purpose of this study is to characterize the percutaneous absorption of two tramadol formulations (Tramadol 100 mg/g in PLO and Tramadol 100 mg/g in Lipoderm), when applied to the inner ear of domestic feline skin, *in vitro,* using the Franz skin finite dose model. In domestic felines, transdermal delivery usually involves the rubbing of medication to the animal's inner ear skin surface, a location that cannot be licked and is usually hairless [12]. The Franz skin finite dose model has proven to be a valuable tool in predicting *in vivo* percutaneous absorption of topically applied drugs [11,13]. Therefore, this model was selected to characterize the percutaneous absorption of tramadol into and through the *ex vivo* inner ear skin of domestic feline donors.

Materials and methods

Materials

Details relating to the composition of the samples used for testing are shown in **Table 1**.

Table 1. Composition of the samples used for testing.

Tramadol 100 mg/g in PLO			
Ingredients	**Quantity**	**Lot number**	**Source**
Tramadol HCl	10 g	C129073	PCCA
Propylene glycol	8 g	C129628	PCCA
Lecithin isopropyl palmitate base	22 g	2007156	PCCA
Poloxamer 407 20% gel	q.s. to 100 g	1722672	PCCA
Tramadol 100 mg/g in Lipoderm			
Tramadol HCl	10 g	C129073	PCCA
Propylene glycol	8 g	C129628	PCCA
Lipoderm	q.s. to 100 g	2039478	PCCA

Tramadol 100 mg/g in PLO

Tramadol hydrochloride (HCl), in an amount necessary to result in a concentration of 100 mg/g, was added to Lecithin Isopropyl Palmitate solution base along with 10% Propylene Glycol as a wetting agent. Poloxamer 407 20% gel was then added in sufficient quantity to reach the final weight. The formulation was mixed using an electronic mortar and pestle (EMP) for 3 min on a setting of 7, sheared twice using an ointment mill at a setting of 1, and remixed for 1 min on a setting of 5. The specific gravity of the final formulation was 1.002 g/mL.

Tramadol 100 mg/g in Lipoderm

Tramadol HCl, in an amount necessary to result in a concentration of 100 mg/g, was added to Lipoderm along with 10% Propylene Glycol as a wetting agent. The formulation was mixed with the aid of an EMP for 3 min at a setting of 7, sheared twice using an ointment mill on a setting of 1, and remixed with an

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EMP for 1 min at a setting of 5 to achieve accurate content uniformity. The density of the final formulation was 0.726 g/mL, thus the final tramadol HCl concentration in weight per volume was 72.6 mg/mL.

Skin preparation

The percutaneous absorption of two tramadol formulations were evaluated using normal feline ventral inner ear skin obtained from two different donors, a domestic short hair feline female (donor 1) and male (donor 2). Prior to use, the skin was thawed in water at approximately 37°C and rinsed with tap water to remove blood and other material from the surface. The ventral (inner) surface of the skin was then separated from the dorsal layer. The skin from the two donors were cut into multiple sections to fit nominal 0.8 cm^2 Franz diffusion cells, a chamber that cultures the skin cells and allows for the condition of the skin to be maintained at a temperature and humidity that matches *in vivo* conditions [13]. Seven skin cross-sections were obtained from the two donors. Each skin section was assigned to one chamber, with four chambers for donor 1 and three for donor 2 (**Table 2**). Within the chambers, the cells were mounted on a diffusion apparatus so that the epidermal surface is exposed to the laboratory environment via the chimney (**Figure 1**). The receptor compartment of the chamber was filled to capacity with isotonic Phosphate-Buffered Saline (PBS), pH 7.4±0.1, stirred magnetically at approximately 600 rpm. The skin surface was maintained at a temperature of 32°C±1°C.

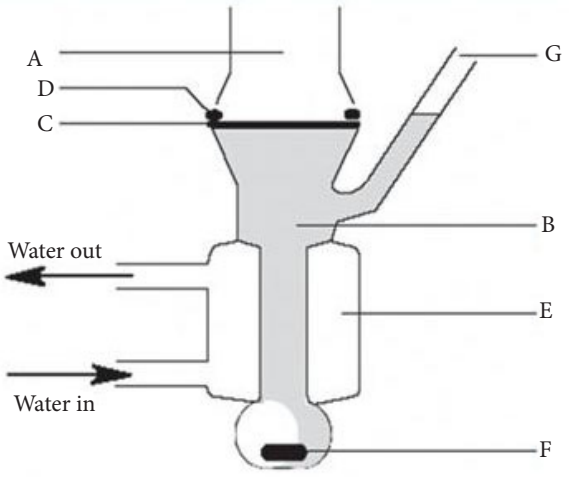
Dosing and sample collection

Prior to dosing, the receptor solution was removed and replaced with fresh solution containing 0.1xPBS with 0.1%

Table 2. Dosing details for the seven chambers.

	Donor 1				Donor 2		
Skin chamber	1	2	3	4	5	6	7
Tramadol in PLO	X	X	--	--	X	--	--
Tramadol in Lipoderm	--	--	X	X	--	X	X

Volpo. The chimney was next removed to allow for access to the epidermal surface for dose application. The two formulations were tested on duplicate sections from two different donors for percutaneous absorption of tramadol over a 48 hr dosing period. Due to the odd number of skin sections available from the two donors, tramadol in PLO was applied to three skin sections (two from donor 1 and one from donor 2) while tramadol in Lipoderm was applied to four skin sections (two from each donor) (**Table 2**). Using the finite dose technique, a variable finite dose (e.g., 25 µg/cm^2) of each formulation was applied to the outer surface of each skin section using a positive displacement pipette set to deliver 5 µL formulation/cm^2 [11]. The dose was then



A. Chamber chimney (open to environment)
B. Receptor solution compartment (4.5-6.5 ML)
C. O-ring seal
D. Skin (1.0cm^2)
E. Heated water jacket
F. Magnetic stir bar
G. Sampling port

Figure 1. Franz diffusion cell.

spread across the surface with a glass rod and the chimney was replaced five to ten minutes after dose application. At 2, 4, 8, 12, 24, 32, and 48 hr following dose application, the receptor solution was removed, replaced with fresh receptor solution, and a predetermined volume of aliquot was saved for subsequent High-Performance Liquid Chromatography (HPLC) analysis. Samples that were not assayed on the day of collection were stored at or below -20°C.

Following collection of the last receptor solution sample at 48 hr, surfaces of the skin were washed twice (0.5 mL each time) with 80:20 Ethanol:Water to remove unabsorbed formulation from skin surfaces. The intact skin was then removed from the chamber and extracted in 80:20 Ethanol:Water overnight at room temperature. Tramadol skin content was determined via HPLC analysis of the extractant sample.

Analytical testing

Tramadol contents within the skin, in the surface wash, and in each receptor solution sample collected at the predetermined time intervals were quantified using HPLC/UV (Ultraviolet). HPLC was conducted on a Hewlett-Packard 1100 Series HPLC system with a diode array detector at a wavelength of 220 ηm (4 ηm) to 500 ηm (50 ηm). A solvent system consisting of 70% Water (pH 9.5) with 10 mM Ammonium Formate and 30% Methanol was run through a Phenomenex Gemini C18 column (3 µ, 50x3 mm) at a flow rate of 0.4 mL/min. The column temperature was maintained at 25°C.

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Results

To characterize the percutaneous absorption of tramadol, a total of 4 parameters were determined for each of the 7 chambers, as follows:total absorption, rate of absorption, surface wash, and skin content. Since each chamber had corresponding values for the 4 parameters, mean values and standard deviation were calculated for each parameter across the 2 donors. Mean values were expressed as percentages of the applied dose to further compare the abilities of PLO and Lipoderm in facilitating the percutaneous absorption of tramadol. Summary of res-ults across the two donors can be found in **Tables 3** and **4** and **Figure 2**.

Total absorption (µg) was calculated as the sum of tramadol content (µg) within the 7 samples collected over 48 hr. Mean total absorption (µg) was the average of total absorptions for all chambers from the 2 donors. The mean total absorption of tramadol in PLO was 333.70 µg±43.09, corresponding to 90.28%±14.70 absorption of the applied dose. For tramadol in Lipoderm, mean total absorption was 277.10 µg±11.39, corresponding to 100.40%±3.24 absorption of the applied dose.

Rate of absorption, presented as flux (µg/cm²/hr) of tramadol into receptor solution, was determined by dividing the amount of tramadol absorbed during a time interval and the length of that interval. Flux is a value reported at midpoint of sample collection for a sampling period. For instance, if samples are collected at 4 and 8 hr, the flux of tramadol during that time interval is equal to the amount of tramadol in the receptor solution between 4 and 8 hr, divided by the time span of 4 hr. Flux is then plotted at 6 hr, midpoint of 4 and 8 hr. Mean flux (µg/cm²/hr), shown in **Table 4**, was calculated across donors for each sampling interval. **Figure 2** illustrates mean flux data plotted as the amount of tramadol absorbed through the skin against time.

Table 3. Across donor summary: mean total absorption, surface wash and skin content (mean±SE (standard error) as percent applied dose, n=2).

	Total absorption (µg)	Total absorption (%)	Surface wash (µg)	Surface wash (%)	Skin content (µg)	Skin content (%)
Tramadol in Lipoderm	277.10±11.39	100.40±3.24	9.86±0.15	3.65±0.14	2.97±0.28	1.09±0.07
Tramadol in PLO	333.70±43.09	90.28±14.70	39.66±54.79	10.45±14.42	<LOD	<LOD

Table 4. Across donor summary: mean flux (µg/cm²/hr) (mean±SE, n=2 donors).

Time (hr)	Tramadol in PLO	Tramadol in Lipoderm
1	17.91±19.00	13.57±3.19
3	15.40±16.28	15.40±0.71
6	10.32±8.70	13.80±0.54
10	10.31±6.68	14.21±1.73
18	12.55±2.78	9.80±1.02
28	6.48±3.93	4.36±1.05
40	4.09±5.01	1.50±0.21

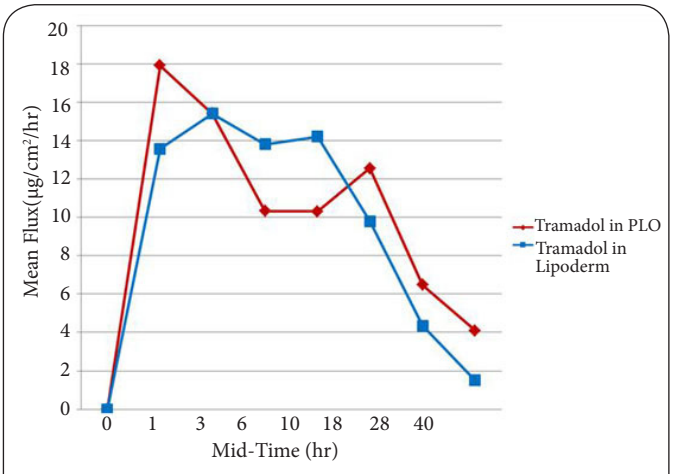

Figure 2. Across donor summary: mean flux (µg/cm²/hr) for two compounded transdermal formulations.

Surface wash (µg) refers to the amount of tramadol remaining on the surface of the skin after 48 hr of dose application. Surface wash was 39.66 µg±54.79 for tramadol in PLO and 9.86 µg±0.15 for tramadol in Lipoderm, corresponding to 10.45% ±14.42 and 3.65%±0.14 of the applied dose, respectively.

Skin content (µg) refers to the amount of tramadol found within the skin after 48 hr. Mean skin content was calculated as the average tramadol content in the skin sections across 2 donors. Mean tramadol skin content was less than the Lower Limit of Detection (LOD) for tramadol in PLO. For tramadol in Lipoderm, mean skin content was 2.97 µg±0.28, which is equivalent to a mean of 1.09%±0.07 of the applied dose.

Discussion

Results of this study show that both PLO and Lipoderm were capable of facilitating the percutaneous absorption of tramadol across *ex-vivo* feline inner ear skin using the Franz skin finite dose model. For both transdermal formulations (tramadol in PLO and tramadol in Lipoderm), the majority of the applied dose penetrated the skin as more than 90% was found in the receptor solution after 48 hr. However, the mean total absorption of tramadol when in Lipoderm (100.40%±3.24) was higher than when in PLO (90.28%±14.70). The higher mean percent total absorption of tramadol seen with Lipoderm potentially indicates that this transdermal base has greater penetration potentials in comparison to PLO. If the tramadol in PLO and tramadol in Lipoderm are both applied to domestic feline inner

ear skin, tramadol plasma concentrations may be higher in domestic felines receiving the Lipoderm formulation.

When examining rate of absorption, mean flux profile reveal similar time course of tramadol absorption for PLO and Lipoderm, with a rapid rise to a peak flux within 2.5 hr of dose application for both transdermal formulations. Though tramadol, when in Lipoderm, had a lower peak flux, the decline in rate was more steady, forming a more predictable and reliable absorption profile in comparison to the rapid decline and fluctuations in rate for tramadol in PLO (**Figure 2**). The steadier decline in flux seen with tramadol in Lipoderm may be helpful when managing domestic feline pain as the analgesic effects of tramadol can slowly taper off overtime rather than having a rapid reduction followed by erratic fluctuations of analgesic effects with PLO.

As for surface wash, tramadol content in the surface wash was lower when in Lipoderm than when in PLO. This difference indicates that more tramadol was absorbed when in Lipoderm. Such results then show that Lipoderm may be more efficient at facilitating the percutaneous absorption of tramadol in comparison to PLO as less tramadol remained on the surface following application of the tramadol in Lipoderm formulation. Surface wash results potentially correlate to the higher total absorption of tramadol seen with Lipoderm.

While very minute amounts of the applied dose were found within the skin, tramadol content in the skin was higher for the Lipoderm than the PLO formulation. The higher tramadol skin content may be due to the ability of Lipoderm to partition into various layers of the skin to a greater extent than PLO. Having higher skin content potentially correlates to a slow and steadier decline in flux as tramadol is slowly released from the skin into receptor solution overtime.

The higher mean total absorption, steadier decline in flux, lower surface wash, and higher skin content seen with the tramadol in Lipoderm formulation show that Lipoderm may be a more appropriate transdermal base for the transdermal delivery of tramadol in comparison to PLO. However, since the study was conducted *in vitro*, future studies need to be conducted *in vivo* to fully inform practitioners and pharmacists on the efficacy of tramadol in Lipoderm compared to tramadol in PLO for domestic feline pain.

Conclusions

Knowledge of this study's results can be used to predict the *in vivo* rate and extent of percutaneous absorption of tramadol in domestic felines. The data collected can augment the limited efficacy data relating to compounded tramadol for transdermal delivery in domestic felines. Based on the *in vitro* results of this study, one may likely hypothesize that tramadol, when in Lipoderm, may potentially have a higher total absorption and a steadier decline in rate of absorption in comparison to when in PLO. The prediction can guide practitioners and pharmacists, when prescribing and compounding with tramadol for feline use, to select a transdermal base with the best and most predictable percutaneous absorption potential.

List of abbreviations

DJD: Degenerative Joint Disease
EMP: Electronic Mortar and Pestle
FDA: Food and Drug Administration
HPLC: High-Performance Liquid Chromatography
IPM: Isopropyl Myristate
IPP: Isopropyl Palmitate
IV: Intravenous
LOD: Lower Limit of Detection
NSAIDs: Nonsteroidal Anti-Inflammatory Drugs
PBS: Phosphate-Buffered Saline
PLO: Pluronic Lecithin Organogel
SC: Subcutaneous
UV: Ultraviolet

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	ASB	DB	CS	HP
Research concept and design	✓	✓	--	--
Data analysis and interpretation	✓	✓	--	✓
Writing the article	✓	✓	--	✓
Critical revision of the article	✓	✓	✓	✓
Final approval of article	✓	✓	✓	✓

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