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Research Article

Skin Delivery and Irritation Potential of Phenmetrazine as a Candidate Transdermal Formulation for Repurposed Indications

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Abstract. Phenmetrazine, a selective dopamine and norepinephrine releaser, previously available as an oral anorectic, is prone to be abused. This study aimed to assess the feasibility of delivering phenmetrazine via the transdermal route for a new indication, while also minimizing its abuse potential. The passive permeation of phenmetrazine through dermatomed human cadaver skin was evaluated using static Franz diffusion cells at 10 mg/mL for the fumarate salt, and at 20, 40, and 80 mg/mL for the free base in propylene glycol for 24 h. Further, oleic acid (5% *w*/*w*), oleyl alcohol (5% and 10% w/w), and lauric acid (10% *w*/*w*) were investigated as chemical permeation enhancers to enhance the delivery. Skin irritation potential was assessed using EpiDerm™ *in vitro* reconstructed human epidermal model. The free base showed superior 24-h delivery (8.13 ± 4.07%, 10.6 ± 2.5%, and 10.4 ± 1.4% for groups with 20, 40, and 80 mg/mL of the free base, respectively) to phenmetrazine fumarate salt (undetectable). The successful screening of effective chemical enhancers, oleyl alcohol (5% and 10% *w*/*w*), oleic acid (5% *w*/*w*), and lauric acid (10% *w*/*w*) resulted in significant enhancement of delivery. The calculated therapeutic relevant flux for the potential indication, attention deficit hyperactivity disorder, 20 μg/cm^2/h was met, where a 24-mg daily dose from a 50-cm^2 patch was projected to be delivered to a 60-kg individual. Irritation study results suggest that formulations with therapeutically relevant delivery are likely to be non-irritant. In conclusion, it is feasible to deliver therapeutically relevant amounts of phenmetrazine via the transdermal route.

KEY WORDS: Chemical permeation enhancers; Phenmetrazine; Transdermal delivery; Skin irritation.

INTRODUCTION

Transdermal delivery of psychiatric drugs has been known to improve use and adherence to drug administration (1). In addition to offering flexibility in tailoring the dose and controlling the duration of the pharmacological effect, the transdermal patch has been recognized as a tamper-deterrent formulation strategy for drugs that possess a high abuse potential (2). The monoamine releaser, phenmetrazine, can selectively induce the release of norepinephrine and dopamine, exerting sympathomimetic effects closely allied to those of ephedrine and amphetamine (3,4). Phenmetrazine hydrochloride tablet (Preludin) was first introduced for weight loss in 1954 in Europe (5) but was associated with a high potential to cause psychoses and addiction (6), and prescription sales slowly declined and it was withdrawn from the market. Phenmetrazine was approved previously as an anorectic as its sole indication. In recent years, monoamine releasers have been under investigation as candidates for the management of cocaine dependence. A dose- and time-dependent manner for cocaine substitution was produced by (+) phenmetrazine in cocaine discrimination assay conducted on Rhesus monkey (7). The efficacy of using phenmetrazine to treat attention deficit hyperactivity disorder (ADHD) has also been of interest (4,8).

Oral solid dosage forms, such as tablets, have been misused for drug abuse and are known to be administered intranasally or intravenously for faster and stronger psychostimulant effects (9). To reduce the abuse potential, prodrugs with a delayed onset of action have been prepared. The prodrug of phenmetrazine, phendimetrazine, was synthesized and found to possess less abuse potential than phenmetrazine, as it delayed the appearance of phenmetrazine in the systemic circulation and exerted a milder stimulant effect following administration (10). However, transdermal delivery of phenmetrazine has not been investigated and has a potential to revive the use of phenmetrazine for a broader scope of indications with higher resistance to formulation tampering

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and subsequently, lower abuse potential (11). This first investigation of utilizing transdermal route to deliver phenmetrazine can help gain distinct advantages over the oral route in terms of bypassing the first-pass effect, sustained drug release, ease of termination, and tamper resistance. In the present study, the feasibility of transdermal delivery of phenmetrazine was established in a liquid formulation composed mainly of the drug and a co-solvent, propylene glycol. The development of a transdermal product of phenmetrazine with minimal abuse potential, as an alternative to oral medication, is our long-term goal and thus, the present study evaluated the delivery of a therapeutically relevant dose, including the exploration of enhancement strategies transferrable to future transdermal patch development. Phenmetrazine could be a promising candidate for transdermal delivery based on its physicochemical properties. It is a small, potent molecule with moderate lipophilicity (logP value at 1.79) and low melting point with the base form existing as a liquid at room temperature.

Transdermal delivery provides many advantages, but the development of transdermal products is primarily limited by the low permeability of the skin due to the barrier function of the stratum corneum, which is the outmost layer of the skin. To overcome this barrier, chemical permeation enhancers have been used in some marketed topical and transdermal products. Among chemical permeation enhancer categories, fatty acids/fatty alcohols have been extensively studied and have effectively promoted percutaneous absorption. The most popular one in this category is oleic acid, an 18-carbon monounsaturated fatty acid, which fluidizes the stratum corneum and/or forms depots in the skin to enhance the transdermal delivery of both lipophilic and hydrophilic compounds (12,13). Oleyl alcohol is the non-polar derivative of oleic acid, which also might function in the same manner (14). Lauric acid is a saturated fatty acid with a 12-carbon atom chain, playing the role of lipid fluidizing to promote the transdermal delivery of anti-estrogen (15). Besides, fatty acids were reported to exhibit synergistic enhancement effects with co-solvents, like propylene glycol, present in transdermal formulations (16). Therefore, the enhancement effects of chemical permeation enhancers on the passive permeation have been investigated for phenmetrazine.

Skin irritation potential is another concern for the development of topical/transdermal formulations. EpiDerm™ skin irritation test using reconstructed human epidermis (RhE) as skin analog shows an excellent *in vitro-in vivo* correlation, fulfills multiple ECVAM validations, and is enlisted in OECD TG 439 guideline for the testing of chemicals (17,18). This is one of the *in vitro* test systems proposed to be the alternative of *in vivo* skin irritation test on rabbits or human volunteers in the preclinical screen. Drawbacks of Draize's rabbit skin irritation study have been surrounding the large variation in results and species difference between human and rabbit, while testing on human volunteers, is difficult (19). RhE has a three-dimensional, multilayered, highly differentiated structure consisting of normal, human epidermal keratinocytes, closely resembling the physiological and biochemical properties of human epidermis *in vivo*. Cell/tissue damage manifested by cell viability, a key event in the chemical-induced skin irritation response, is used as the end point, measured by standardized MTT cell viability assay, with concurrent positive and negative controls to ensure reproducibility. In addition, the measurement of drug permeation coupled with skin irritation study can be realized using manufacturer's custom protocols. Comparison of permeability of *in vitro* human cadaver skin model and RhE as well as a correlation between permeation and irritation potential may be done. Therefore, EpiDerm™ skin irritation study was used here to predict skin irritation of phenmetrazine formulations.

The objective of this study was to establish the feasibility of delivering phenmetrazine via the transdermal route for a new indication, while minimizing its abuse potential. The feasibility of delivering a therapeutically relevant dose, including the exploration of enhancement strategy transferrable to future transdermal patch development as well as the evaluation of its skin irritation potential, was assessed. The incorporation of chemical enhancers can increase the delivery, reduce the residual drug amount in the used formulation, and compensate for the potential flux drop when advancing from a liquid formulation towards the semisolid patch formulation, or when the duration of application needs to be shortened. This first investigation of utilizing transdermal route to deliver phenmetrazine has the potential to revive the use of phenmetrazine for a broader scope of indications with higher resistance to formulation tampering.

MATERIALS AND METHODS

Materials

Phenmetrazine fumarate was kindly provided by Dr. Bruce E. Blough of the Research Triangle Institute (Research Triangle Park, NC). Dermatomed human cadaver skin was obtained from the New York Firefighter Skin Bank (New York, NY). Propylene glycol was purchased from EK Industries (Joliet, IL). Sodium hydroxide, sodium phosphate monobasic and dibasic, phosphate-buffered saline, and orthophosphoric acid (85%) were purchased from Fisher Scientific (Bridgewater, NJ). Methanol (HPLC grade) and ethyl acetate (HPLC grade) were obtained from MedSupply Partners (Atlanta, GA). EpiDerm™ skin irritation test kit was procured from the MatTek Corporation (Ashland, MA).

Methods

Preparation of Phenmetrazine Free Base

The extraction of phenmetrazine free base was achieved using liquid-liquid extraction. Phenmetrazine fumarate was completely dissolved in deionized water, followed by pH adjustment with 1 M sodium hydroxide solution to pH value higher than 12.0. The same volume of ethyl acetate was added, and vortex mixed. Then, the mixture was centrifuged at 3000×*g* for 10 mins at room temperature. The supernatant was transferred to a 10-mL round-bottom flask. Ethyl acetate extraction was performed three times, and the free base-containing supernatants were pooled together for subsequent drying using a rotary evaporator (Rotavapor R-3000; Buchi Analytical Inc., Switzerland) in a water bath maintained at 40 °C. The flask was left under vacuum overnight to remove residual ethyl acetate.

Formulation Preparation

Formulations of phenmetrazine were prepared in propylene glycol (PG) as the vehicle alone or in combination with chemical permeation enhancers, oleic acid, oleyl alcohol, or lauric acid at different concentrations. The formulation comprising of PG and oleic acid, oleyl alcohol, or lauric acid was prepared weight by weight before mixing with the drug. The composition of all formulations prepared is listed in Table I. The color and appearance of the formulations were examined visually. The drug content was confirmed with the HPLC-UV method described in the "Analytical Method" section.

Analytical Method

The quantification of phenmetrazine was achieved using Waters Alliance 2695 separation module (Milford, MA) coupled with a 2996 photodiode array detector. The separation was carried out on a Waters XTerra RP_{18} column (3.5 μm, 150 × 4.6 mm) maintained at 35 °C. An isocratic profile was applied with methanol and sodium phosphate buffer (12.5 mM, pH 6.0) at a ratio of 25:75. Thirty microliters of sample were injected. The isocratic elution was performed at the flow rate of 0.8 mL/min with a run time of 10 mins. Data were collected at the wavelength of 211 nm for the analyte.

In Vitro Permeation Study

In vitro permeation studies ($n = 4$–6) were carried out on dermatomed human skin using static Franz diffusion cells. The receptor compartment was maintained at 37 °C by the water jacket. In the receptor compartment, sink condition was maintained using phosphate-buffered saline (PBS, 10 mM, pH 7.4, 5.0 mL) as the receptor solution. The skin was mounted on the surface of the receptor compartment with an exposed area of 0.64 cm^2. The donor compartment was placed on the skin and clamped with the receptor compartment in alignment. The intactness of skin barrier was demonstrated by measuring the skin resistance before the formulation application using silver/silver chloride electrodes, Agilent 33220A, 20-MHz function/arbitrary waveform generator, and 34410A 6½ digit multimeter (Agilent Technologies, Santa Clara, CA). The multimeter display value (voltmeter-measured voltage, V_s) was recorded, and skin resistance (R_s) was calculated using the equation:

$$R_s = \frac{V_s R_L}{(V_0 - V_s)}$$

where R_L, the resistance of the nominal load resistor, and V_0, the source voltage, were 100 kΩ and 100 mV, respectively. Skin pieces with a resistance lower than 10 kΩ were rejected in the study (20). The formulation (100 μL) was placed on the skin surface. Samples of receptor solution (300 μL) were collected at predetermined time points (0, 1, 2, 4, 6, 8, 22, and 24 h) and were replaced with an equal volume of fresh receptor solution. Samples were analyzed using the HPLC-UV method described above.

Skin Irritation Studies on In Vitro Reconstructed Human Epidermal Model

To test the irritation potential of the enhancer-containing formulation, formulation F7 was subject to the EpiDerm™ skin irritation test (EPI-200-SIT) with the three-dimensional *in vitro* reconstructed epidermis model (MatTek Corporation, Ashland, MA) following the manufacturer's instructions (21). The test formulation, positive control (5% sodium dodecyl sulfate), and negative control (PBS) were applied to the reconstructed skin tissue (30 μL, $n = 3$) for 35 mins. After removing the formulation, the treated tissues were washed with PBS and transferred to fresh media for incubation for 24 h. Then, another medium change was performed and followed by 18 h more incubation. To evaluate the tissue viability, the tissues were placed into methyl thiazolyl tetrazolium solution and incubated for 3 h. All incubations were performed at 37 °C in 5% CO_2 incubator. At the end of the incubation, the formazan formed by living cells were extracted using 2 mL of isopropyl alcohol by shaking at 120 rpm for 2–3 h. Aliquots of the formazan extracts (100 μL, $n = 2$) were transferred to a 96-well plate, and the optical density was measured at 570 nm by a Synergy HT plate reader (BioTek Instruments, Inc., Winooski, VT).

Skin Irritation and Permeation Studies on In Vitro Reconstructed Human Epidermal Model

To test the irritation potential as well as the permeation of the drug, formulation F2 was tested following a modified skin irritation test protocol based on manufacturer's instructions. The test formulation, positive control, and negative control were exposed to the reconstructed skin tissue (30 μL, $n = 3$) for 25 h. The following washing and incubation procedures were the same as described above for skin irritation studies on *in vitro* RhE model. Medium samples for the evaluation of phenmetrazine permeation from formulation F2 across the RhE model were taken at 1, 2, 4, 22, and 25 h post-application and analyzed using the HPLC-UV method. Calibration standards were prepared in the same matrix.

Statistical Analysis

Data ($n \geq 3$) were presented as mean ± standard deviation (SD), unless specified otherwise. All the graphs and statistical analysis (*t* test and one-way ANOVA) were generated using GraphPad Prism6 (GraphPad Software, San Diego, CA). Values of $p < 0.05$ were considered as statistically significant.

RESULTS

Preparation of Phenmetrazine Free Base and Formulations

The clear colorless and odorless liquid was obtained for phenmetrazine free base. Vacuum evaporation of residual extraction solvent overnight did not cause appreciable volume loss, indicating no or minimal volatility of phenmetrazine free base at room temperature. Phenmetrazine fumarate dissolved entirely in PG at the concentration of 10 mg/

Table I. The Composition and *In Vitro* Permeation Parameters of Phenmetrazine Formulations

Drug	Phenmetrazine fumarate	Phenmetrazine free base							
Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
API	1% *w/v*	1.5%	2%	4%	4%	4%	4%	4%	8%
Propylene glycol (PG)	99%	98.5%	98%	96%					92%
PG + 5% *w/w* Oleic acid					96%				
PG + 5% *w/w* Oleyl alcohol						96%			
PG + 10% *w/w* Oleyl alcohol							96%		
PG + 10% *w/w* Lauric acid								96%	
Steady-state flux (μg/cm^2/h) ± SE	N/A[a]	7.88 ± 0.15[b]	13.2 ± 6.5	32.6 ± 7.6	136 ± 5	211 ± 11[d]	214 ± 2	59.9 ± 3.6	62.0 ± 8.5
Lag time (h) ± SE	N/A[a]	0.58 ± 0.01	6.21 ± 0.68	3.86 ± 0.10	1.27 ± 0.16	1.77 ± 0.69[d]	1.14 ± 0.29	3.62 ± 0.68	2.96 ± 0.28
% delivery ± SE	N/A[a]	14.4 ± 0.2[c]	8.13 ± 4.07	10.6 ± 2.5	48.5 ± 1.4	72.5 ± 2.5[d]	76.6 ± 0.6	19.4 ± 1.6	10.4 ± 1.4

All formulations were prepared in volume (*v/v*), unless specified otherwise
[a] N/A denotes not available
[b] Steady-state flux was obtained from the *in vitro* RhE model instead of dermatomed human cadaver skin
[c] Data was obtained after 25 h of permeation instead of 24 h
[d] Data was normalized to formulation F5. The normalization factor was the ratio of formulation F5 steady-state flux values in two separate *in vitro* permeation studies using different skin resources. The steady-state flux, lag time, and percentage of delivery values for formulation F6 (non-normalized, $n = 4$) were 188 ± 11, 1.77 ± 0.69, and 64.6 ± 2.2, respectively. The repeated formulation F5 ($n = 4$) from which the steady-state flux was used for normalization had steady-state flux, lag time, and percentage of delivery values of 121 ± 6, 5.20 ± 0.40, and 35.8 ± 2.3, respectively

mL after overnight shaking. Phenmetrazine free base was fully miscible with PG at 15, 20, 40, and 80 mg/mL and the blank blend of PG and chemical permeation enhancers at the drug concentration of 40 mg/mL. All the resulted formulations (as shown in Table I) were clear solutions. The drug concentration in the formulations was confirmed by quantification using the HPLC-UV method prior to the permeation studies.

In Vitro Permeation Study

Passive Permeation of Phenmetrazine

Passive delivery of phenmetrazine fumarate (10 mg/mL) in PG across dermatomed human skin was explored. Over 24 h, no detectable phenmetrazine was observed in the receptor solution.

To evaluate the passive permeation of phenmetrazine free base, formulations of phenmetrazine free base were prepared in PG at three concentrations, 20, 40, and 80 mg/mL, in which *in vitro* permeation studies across the dermatomed human skin were carried out. The cumulative amount of drug permeated through the skin was plotted *versus* time in Fig. 1. After 24 h, a considerable amount of drug was observed in the receptor solution from all the groups. Steady-state flux was obtained from the slope of the linear equation after curve fitting of the graph plotting cumulative amount against time. The *X*-intercept from the linear equation was considered as lag time. The percentage of delivery was calculated by comparing the cumulative amount to the drug applied in the donor compartment (assuming 100%). The cumulative amount and steady-state flux were found to be dose proportional. Lag time for the 20-mg/mL group was significantly ($p < 0.01$) longer than two higher dose groups. The 40-mg/mL group has met the calculated therapeutically relevant flux level, 20 μg/cm^2/h. In addition, interpolation from the linear regression ($Y = 0.802 \times X$-1.49, $r^2 = 0.995$) of a plot with steady-state flux drawn against dose level, resulted in a dose level of 26.8 mg/mL for achieving a steady-state flux of 20 μg/cm^2/h. Across the dose range, the percentage of delivery remained to be ~ 10%, indicating a considerable amount of drug was remaining in the formulation after the 24-h permeation study.

Influence of Chemical Permeation Enhancers on the Permeation of Phenmetrazine Free Base

The *in vitro* permeation of phenmetrazine with the incorporation of chemical permeation enhancers was further investigated and compared with PG only group ($n = 4$ for each group). Chemical permeation enhancers belonging to the fatty acid/fatty alcohol groups, such as oleic acid, oleyl alcohol, and lauric acid, were screened. The concentration used was within the ranges in the inactive ingredient list for approved drug products or published report (15,22). The enhancement effect was first explored with 5% *w/w* oleic acid (F5), 10% *w/w* oleyl alcohol (F7), and 10% *w/w* lauric acid (F8). The cumulative drug amount and the corresponding flux were plotted *versus* time in Fig. 2. As shown in the permeation profile, all the tested chemical permeation enhancers significantly ($p < 0.01$) improved the cumulative amount of phenmetrazine at 24 h as compared to the enhancer-free group. Oleic acid (5% *w/w*) and oleyl alcohol (10% *w/w*) significantly ($p < 0.0001$) increased the percentage of delivery to 48.5 ± 1.4 and 76.6 ± 0.6, respectively, and thereby only ~ 25% of phenmetrazine was remaining in the donor compartment with the addition of oleyl alcohol. The flux value for each time point in the flux profile represents the amount of drug permeated over the time period between the current and previous sampling points. Tested enhancers remarkably accelerated the permeation flux until 6 or 8 h, while unlike the PG only group, the

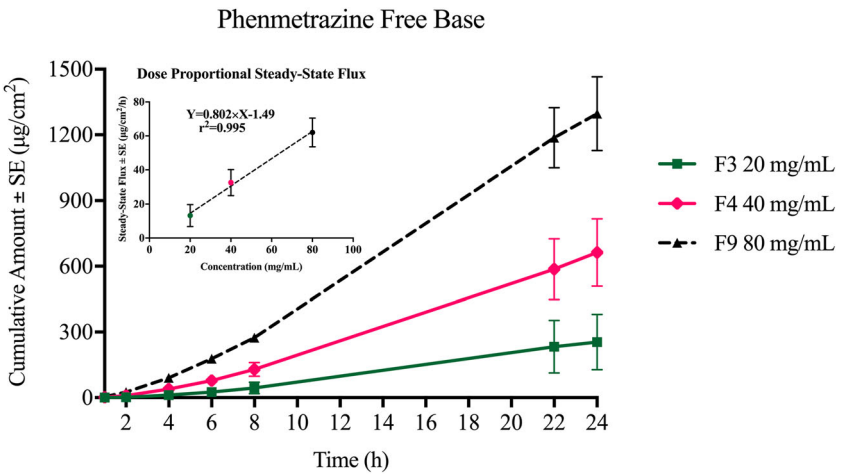

Fig. 1. The *in vitro* permeation profiles of phenmetrazine free base at 20, 40, and 80 mg/mL in PG.

flux for formulation with enhancers dropped after that, possibly resulted from the decrease of drug concentration in the donor compartment. Compared to the PG only group (replicates of four using the skin from the same skin donor were counted), oleyl alcohol (10% *w/w*), oleic acid (5% *w/w*), and lauric acid (10% *w/w*) enhanced the steady-state flux of phenmetrazine significantly for 10.0-fold ($p < 0.0001$), 6.4-fold ($p < 0.0001$), and 2.8-fold ($p = 0.02$), respectively. A significant less lag time was observed for oleic acid (5% *w/w*) and oleyl alcohol (10% *w/w*) groups ($p = 0.03$ and 0.04, respectively). Overall, oleyl alcohol (10% *w/w*) exhibited the most profound enhancement effect for phenmetrazine free base among the enhancers tested.

Influence of 5% w/w Oleic Acid and 5% w/w Oleyl Alcohol on the Permeation of Phenmetrazine Free Base

The two most effective enhancers, oleic acid and oleyl alcohol, were then studied at different concentrations. To further elucidate the result, the influence of 5% *w/w* of oleyl alcohol and 5% *w/w* of oleic acid on the drug permeation was compared in a separate *in vitro* permeation study, in which 5% *w/w* of oleic acid (F5) group was repeated. The concentration of oleyl alcohol was lowered to be 5% *w/w* (F6) for direct comparison. To also allow for the comparison between 5% *w/w* and 10% *w/w* of oleyl alcohol groups, the cumulative amount from the 5% *w/w* of oleyl alcohol group (F6) was normalized to the previous study where the groups of 10% *w/w* of oleyl alcohol (F7) and 5% *w/w* of oleic acid (F5) were first investigated. The ratio of steady-state flux between the groups with 5% *w/w* of oleic acid in both studies was used for normalization. Hence, the normalized permeation and flux profiles of F6 were incorporated into Fig. 2. Significantly higher cumulative amount ($p < 0.0001$), steady-state flux ($p < 0.0001$), and percentage of delivery ($p = 0.0012$) were found in the 5% *w/w* of oleyl alcohol group than those in the 5% *w/w* of oleic acid group. However, there was no significant difference between 5% *w/w* and 10% *w/w* of oleyl alcohol groups in the extent or rate of phenmetrazine delivery, indicating oleyl alcohol at both 5% *w/w* and 10% *w/w* was equivalently effective but more effective than oleic acid at 5% *w/w*.

Skin Irritation Studies on *In Vitro* Reconstructed Human Epidermal Model

To predict the skin irritation potential of phenmetrazine, we have exposed the formulation having the highest delivery rate (steady-state flux = 214 ± 2 μg/cm^2/h) to the *in vitro* RhE model. Formulation F7 consisted of 40 mg/mL of phenmetrazine free base and 10% *w/w* of oleyl alcohol in PG. Tissue viability for formulation F7 relative to the negative control group was lower than 50%, as shown in Fig. 3. Therefore, formulation F6 was considered an irritant. The steady-state flux of formulation F7 was obtained from *in vitro* permeation studies across dermatomed human cadaver skin, which was ~ ten-fold higher than the required flux (20 μg/cm^2/h). At flux levels close to the calculated required flux, skin irritation is less likely to happen. Since the actual flux across the *in vitro* RhE tissue remained unknown, we further coupled the skin irritation test with the permeation study to enable a direct correlation between the skin irritation and flux.

Skin Irritation and Permeation Studies on *In Vitro* Reconstructed Human Epidermal Model

Formulation F2 with 15 mg/mL of phenmetrazine free base was used in this study as the permeability of the *in vitro* RhE model may be higher. The formulation was left on the RhE tissue for 25 h and samples from the media were taken until the end of the formulation exposure. The permeation profile is shown in Fig. 4a. Steady-state permeation flux (7.88 μg/cm^2/h) was obtained between 1 and 8 h, and the lag time was 0.58 h. At the same dose level, the steady-state flux across the RhE model was found to be similar to the predicted steady-state flux across dermatomed human skin (10.5 μg/cm^2/h) that was extrapolated from the equation shown in Fig. 1, suggesting comparable permeability between two skin models. After 25-h exposure of phenmetrazine formulation, the relative tissue viability was found to be 81.8 ± 15.7% (Fig. 4b), which was classified as not irritant, implying that a therapeutically relevant dose is likely to be delivered safely through the skin.

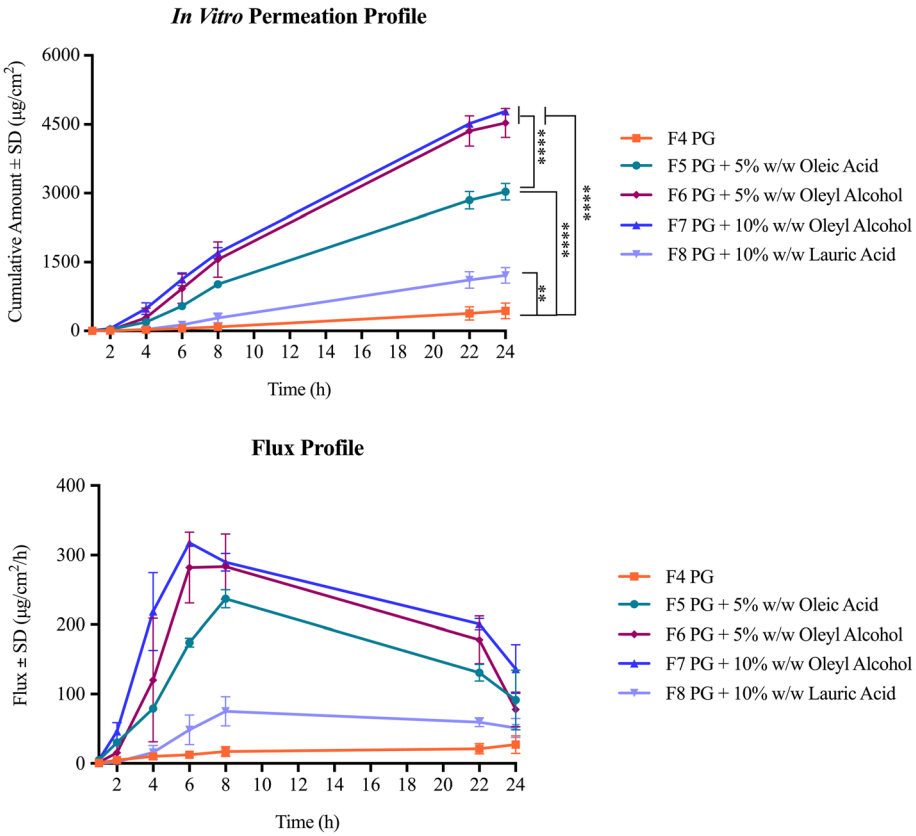

Fig. 2. The *in vitro* permeation profiles: cumulative amount and permeation flux of phenmetrazine free base at 40 mg/mL in PG, and PG with 5% *w/w* oleic acid, 5% *w/w* and 10% *w/w* oleyl alcohol, and 10% *w/w* lauric acid. (One-way ANOVA: $*p < 0.05$, $**p < 0.005$, $***p < 0.001$, $****p < 0.0001$)

DISCUSSION

Aiming to develop phenmetrazine transdermal patches in the long term, we investigated the feasibility of delivering phenmetrazine via passive diffusion for transdermal delivery.

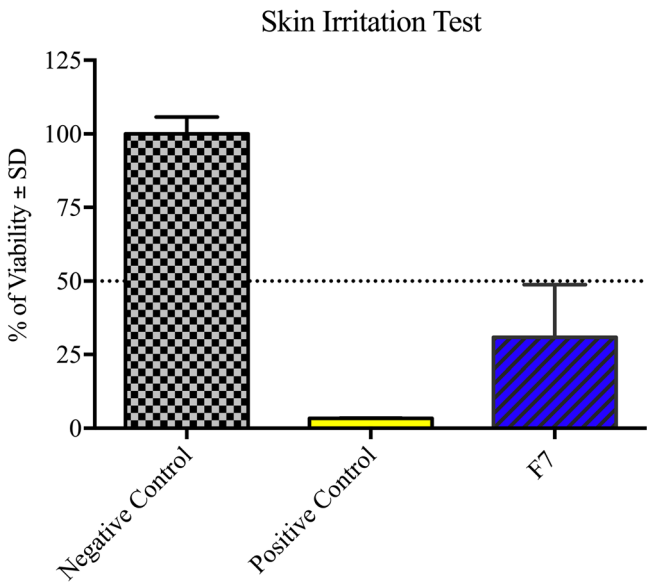

Fig. 3. Skin irritation test for formulation F7 (40 mg/mL of phenmetrazine free base in PG with 10% *w/w* of oleyl alcohol, $n = 3$)

In vitro permeation studies across dermatomed human skin using Franz diffusion cells were performed. In these studies, PG was used as the vehicle because it could readily permeate the skin and carry the drug molecules along (23), which may maximize the extent of passive delivery of a compound. The result of *in vitro* permeation study with the salt form suggests that phenmetrazine fumarate, or phenmetrazine salts formed with other acid species, might not be good candidates for passive transdermal delivery since the charged form of molecules are not preferred for diffusing across the lipophilic skin barrier. The computed logP value of phenmetrazine was in the optimum range (logP = 1–3) for passive transdermal diffusion, however, was for the unionized/uncharged free base form. The uncharged form of the drug is widely used as the active pharmacological ingredient in marketed patch products via passive diffusion. Diclofenac epolamine patch (Flector®) is available for local pain relief. Diclofenac acid has logP at 4.5, while diclofenac epolamine has logD at 3.22 and 1.10 when pH is 5 and 7.4, respectively (24). In the case of diclofenac acid, the formation of salt is positively influencing the logD to be in the favorable range for passive permeation. The higher aqueous solubility of the salt also promotes the partition of the drug out of stratum corneum to the systemic circulation (25). However, the phenmetrazine fumarate salt may have lowered its logD to be more hydrophilic or below the favorable hydrophobicity range. The logD of the hydrochloride salt of phenmetrazine is − 1.14 and 0.98 at pH 5 and pH 7.4, respectively. Therefore, further studies were performed with the free base form of phenmetrazine.

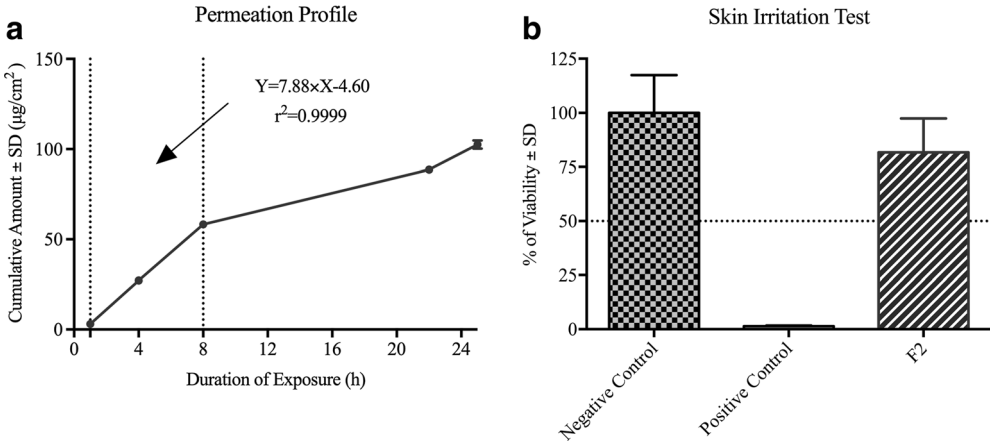

Fig. 4. The *in vitro* permeation profile (**a**) and skin irritation test (**b**) for formulation F2 (15 mg/mL of phenmetrazine free base in PG, $n = 3$) on *in vitro* reconstructed human epidermal model

In vitro permeation results suggest that the base form of phenmetrazine is a promising candidate for transdermal delivery. Assuming a constant delivery over the duration of application, the calculated therapeutic relevant flux for ADHD would be 20 μg/cm^2/h, which shall ensure a 24-mg dose delivered from a 50-cm^2 patch to a 60-kg individual per day. The transdermal target dose was calculated based on our collaborator's unpublished preliminary data, in which an intramuscular bolus injection of 0.1 mg/kg of phenmetrazine improved attention in a gold standard non-human primate (Rhesus Macaques) model. The predictive validity of this primate model of attention greatly supports the potential repurposing of phenmetrazine for ADHD treatment. Since the duration of the behavioral test was 1 h post-administration, we projected that a constant dose of 0.1 mg/kg/h is a good estimate of an appropriate dose. The FDA guidance is to use interspecies dose scaling to divide a primate dose by 3.1 to determine the human equivalent dose (given the larger size and slower metabolism of humans, among other factors). 0.1 mg/kg divided by 3.1 yields a human equivalent dose of 0.032 mg/kg/h. As the ADHD indication will primarily target adolescents (and the cocaine-use disorder indication will likely target underweight adults given their chronic use of stimulants and poor health status), we assumed a human patient body weight of 60 kg. 0.032 mg/kg/h multiplied by 60 kg equals 1.94 mg/h in humans. Further, the bioavailability factor was taken into consideration. Although intramuscular injection can lead to high absolute bioavailability, we assumed the intramuscular bioavailability to be 50% because the reported oral bioavailability was only 1% (26). 1.94 mg/h multiplied by 50% yields a bioavailability normalized target dose of 0.97 mg/h. We therefore targeted a dose of 24 mg/day as that corresponds to the duration of *in vitro* permeation study, 24 h. However, we expect that patients will wear the patch from 9 h (primarily during school for ADHD as is done with Daytrana) to 15 h (all day except when sleeping for the cocaine-use disorder) per day. To corroborate this dose range, we matched the target dose with the highest strength of an extended-release amphetamine tablet for ADHD. Adderall contains 3.13–18.8 mg of total amphetamine base equivalence. We felt that this corroborated our dose range of 24 mg/day. The target dose could be met; however, the transition from a liquid formulation to a semi-solid transdermal patch formulation may result in a decrease in the steady-state flux. To ensure a therapeutically relevant delivery for any condition that phenmetrazine may be indicated for, further improvement in the steady-state flux of phenmetrazine based on the 40-mg/mL group results was investigated.

Albeit using the formulation with a higher concentration of drug is highly likely to improve the flux further and thus increase the delivery from the same application area, the residual drug amount in the formulation may increase in that case, because the percentage of delivery of the drug does not necessarily increase as the drug concentration increases. Phenmetrazine is a schedule II-controlled substance in the USA. The disposal of medications as such has always been a concern to both health and environment. A large portion of the drug remained unabsorbed in all the enhancer-free groups but preferred to be lowered. To enhance drug delivery as well as reduce remaining drug, chemical permeation enhancers, an enhancement technique which is highly amenable to transdermal patch formulation environment, therefore, has been explored. They are usually pharmaceutically inactive and may modify the stratum corneum by one or more of three primary mechanisms: (a) disruption of the highly ordered structure of stratum corneum lipid; (b) interaction with intercellular protein; (c) improved partition of the drug, co-solvent, or enhancer into the corneum (27). Fatty acid/alcohol is a class of chemical permeation enhancer that fluidizes and forms depot in the skin to promote the percutaneous delivery of drugs. The enhancement effect of fatty acids depends on the structures, alkyl chain length, and saturation degree (28–30).

All tested chemical enhancers worked but differed in the magnitude of enhancement effect. Theoretically, the fraction of ionization of the drug is not expected to change in propylene glycol. When there is a pH change after adding ionizable permeation enhancers, namely oleic acid and lauric acid, the free base is likely to form ion pairs with the enhancers, which might have slowed down the permeation of phenmetrazine by decreasing the available concentration of the favorable uncharged form in the donor compartment.

Concomitantly, ionized chemical enhancer may undergo less partition into the stratum corneum, and thus become less effective (*e.g.*, oleic acid *vs*. oleyl alcohol). Moreover, the free base form is highly likely to be used in the transdermal patch development, for which the pH of the environment is not controlled. Therefore, the enhancer-containing groups were tested without any pH adjustment.

Skin safety during application is as important as sufficient delivery. To predict the skin irritation potential of phenmetrazine, we have exposed the *in vitro* RhE model to the formulation having the highest delivery rate across human cadaver skin. Formulation F7 was considered an irritant. The irritancy could be due to both the high steady-state flux and the incorporation of permeation enhancers. The skin irritation potential is likely to be flux-dependent, as reported for nicotine transdermal patches (31). Also, the addition of oleyl alcohol may have contributed to the skin irritation as well, though it was used within the safe range. Formulation F2 with 15 mg/mL of phenmetrazine free base was used in the study where both skin irritation and permeation were evaluated. A lower concentration was used because the permeability of RhE model was initially hypothesized to be higher, though found to be similar to that of human cadaver skin later. Therefore, a lower concentration that was presumed to show the target steady-state flux across RhE was used in the coupled *in vitro* permeation and irritation study to predict the drug's skin irritation potential. Formulation F2 showed no irritancy and resulted in a steady-state flux of 7.88 $\mu g/cm^2/h$ on the RhE model. Though the value was lower than the target steady-state flux, formulation F2 was expected to be able to deliver a dose in the therapeutically effective range, given that a sufficiently large area of application is used. This again proves that at therapeutically relevant flux, the drug is likely to be delivered safely through the skin.

CONCLUSION

In conclusion, the *in vitro* permeation study and irritation study results suggest that it is feasible to deliver phenmetrazine via transdermal route. The incorporation of chemical enhancers in the formulation was able to modulate the transdermal delivery of phenmetrazine, among which oleyl alcohol was the most effective, with no significant difference found in the enhancement effect using 5% w/w and 10% *w*/*w*. For future perspective, formulations with therapeutically relevant flux are highly likely to be non-irritant to the skin, and the development of a transdermal product with reduced abuse potential of phenmetrazine holds promise.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest The authors declare that they have no conflicts of interest.

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