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RESEARCH PAPER

Effect of Controlled Heat Application on Topical Diclofenac Formulations Evaluated by *In Vitro* Permeation Tests (IVPT) Using Porcine and Human Skin

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ABSTRACT

Purpose Heat therapy is widely used for pain relief and may unintentionally be used in conjunction with pain relieving topical formulations. The purpose of this study was to evaluate the influence of heat on the permeation of diclofenac through porcine and human skin, comparing four marketed products.

Methods *In vitro* permeation tests (IVPT) were performed on porcine skin from a single miniature pig and human skin from three donors. Skin temperature was maintained at either 32 ± 1°C or 42 ± 1°C to mimic normal and elevated skin temperature conditions, respectively.

Results IVPT studies on porcine and human skin were able to demonstrate heat-induced enhancement in flux and cumulative amount of drug permeated from the four diclofenac products. The pivotal data showed the most significant heat-induced enhancement for the solution, followed by the patch and two gels in decreasing order of significance based on *p*-values. Diclofenac solution showed the highest flux and cumulative amount permeated at both baseline and elevated skin temperature compared to the patch and gels.

Conclusions The studies demonstrated that exposure to heat can alter drug permeation from topical formulations, but the increased levels are not expected to lead to systemic concentrations that are of concern. Formulation design and excipients can influence drug permeation at elevated skin temperature.

KEY WORDS diclofenac · drug permeation · heat · temperature

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ABBREVIATIONS

ANOVA	Analysis of variance
API	Active pharmaceutical ingredient
CHADD	Controlled heat-aided drug delivery
CHTN	NCI Cooperative Human Tissue Network
C_{max}	Maximum concentration
CV	Cardiovascular
HA	Hyaluronic Acid
HPLC	High-performance liquid chromatography
IVPT	*In vitro* permeation test
J_{max}	Maximum flux
LLE	Liquid-liquid extraction
LLOQ	Lower limit of quantification
logP	Logarithm of octanol-water partition coefficient
pKa	Acid dissociation constant
QC	Quality control
SC	Stratum corneum
TEWL	Transepidermal water loss

INTRODUCTION

The topical drug delivery market in North America is growing at a compound annual growth rate of 8% and is projected to double its market value by 2024 compared to 2017, according to DataBridge Market Research. Topical formulations have several advantages including ease of administration and local action with minimal systemic effects. Pharmacokinetic characteristics of some of these products under optimal conditions are provided on the package insert. However, several external factors such as exposure to heat, humidity, and occlusion can alter drug release and permeation (1,2). Therefore, it is possible that these environmental factors can alter the pharmacokinetic profile and influence safety and clinical efficacy of transdermal and topical drug products. Heating pads and electric blankets are widely used for pain relief and to provide warmth, respectively. Their

unintentional application simultaneously with a transdermal or topical system can result in an unexpected increase in drug delivery (3,4). Other sources of heat that can potentially elevate skin temperature include saunas, hot tubs, and exercise. *In vivo* application of heat has been shown to result in increased plasma levels of fentanyl, nicotine, nitroglycerin, and glyceryl trinitrate (5–10). Use of controlled heat-aided drug delivery (CHADD) systems that utilize controlled heat to aid transdermal delivery of fentanyl, lidocaine, and testosterone significantly increased plasma levels of these drugs (11–13). Studies investigating the influence of heat on topically applied formulations were focused on patch evaluation with a few exceptions (5,6,9,13,14). For example, the effect of heat when evaluated for lidocaine, caffeine, methyl, and butyl paraben suspensions indicated a significant increase in flux at 45°C (15). Likewise, an increase in acyclovir release from Zovirax® cream was reported *in vitro* when the temperature was increased to 37°C (16). There are a few reports investigating the influence of heat application on semisolid formulations. For example, heat application on the skin of human subjects prior to the application of Voltaren® gel showed no significant increase in diclofenac permeation (17).

Heat is expected to induce enhancement of drug release and permeation which can be attributed to several factors, including increased drug release from the formulation, increased stratum corneum fluidity, enhanced diffusivity of solute through skin (as suggested by the Arrhenius equation), and increased dermal clearance due to enhanced skin perfusion (18,19). Study designs including heat exposure on formulation as well as the skin are therefore expected to have a greater influence on drug permeation due to the elevated temperature. Most semisolid topical agents are locally acting and hence these formulations are not designed to achieve high systemic drug levels, thus having a better systemic safety profile than their oral counterparts. However, higher than expected systemic drug levels may be reached upon exposure of topical formulations to immoderate conditions. Patients with known cardiovascular (CV) disease or its risk factors are more sensitive to elevated levels of diclofenac and hence more prone to CV adverse effects (20). Heat can influence products with the same active pharmaceutical ingredient (API) but different inactive ingredients to a similar extent or to different extents. A study on two types of fentanyl patches showed a two-fold increase in *in vitro* release profiles under heat exposure (7). The magnitude of heat-induced enhancement in drug permeation can be affected by the physical and chemical nature of the formulation, drug load, and physicochemical properties of the drug molecule. This can result in altered pharmacokinetic profiles in both patch and semisolid topical or transdermal formulations.

The purpose of this study was to evaluate the influence of heat on the permeation of a model lipophilic, weakly acidic drug, diclofenac (logP = 4.51 and pKa = 4.15), through human and porcine skin compared among four topical products (diclofenac epolamine 1.3% patch, diclofenac sodium 2% solution, diclofenac sodium 1% gel, and diclofenac sodium 3% gel) (Table I) using IVPT. *In vitro* flux profile data can provide a mechanistic understanding of heat effect on topical formulations. Our aim was not to de-convolute the complicated mechanisms involved in heat-induced drug delivery at this stage in the project, but to determine whether or not heat application with topical formulations could increase systemic drug levels. A central consideration in the study was to evaluate if heat application could have significantly different influences on products with the same API but diverse inactive ingredients.

MATERIALS AND METHODS

Materials

Flector® 1.3% patch (lot # 1205142, exp. 05/2015 and lot # 1601138, exp. 01/2019), Pennsaid® 2% solution (lot # P0871A, exp. 12/2015 and lot # U1000A, exp. 12/2017), Voltaren® 1% gel (lot # W3686, exp. 05/2016), and Solaraze® 3% gel (lot # 3098701, exp. 02/2015) diclofenac

Table I Comparison of the Four Topical Diclofenac Products

	1.3% Patch	2% Solution	1% Gel	3% Gel
Inactive ingredients	Adhesive in an aqueous base containing sodium polyacrylate, sodium carboxymethylcellulose	DMSO, ethanol, purified water, propylene glycol, hydroxypropyl cellulose	Carbomer homopolymer Type C, cocoyl caprylcaprate, fragrance, isopropyl alcohol, mineral oil, polyoxyl 20 cetostearyl ether, propylene glycol, purified water, strong ammonia solution	Hyaluronate sodium, benzyl alcohol, polyethylene glycol monomethyl ether, purified water
Dose applied	0.97 cm^2	5 mg/cm^2	10 mg/cm^2	20 mg/cm^2
Equivalent amount of diclofenac in applied dose	928 $\mu g/cm^2$	93 $\mu g/cm^2$	93 $\mu g/cm^2$	558 $\mu g/cm^2$

topical products were purchased from Cardinal Health™ (Dublin, OH). Potassium phosphate monobasic and dibasic salts, methanol, acetonitrile, ethyl acetate, and ethanol were purchased from Fisher Scientific Inc. (Fair Lawn, NJ). Phosphoric acid (95%) was purchased from EMD Millipore (Billerica, MA). Flufenamic acid was purchased from Sigma-Aldrich (St. Louis, MO). Diclofenac sodium salt (≥98%) was obtained from TCI America (Portland, OR). All reagents used were of analytical grade. Water filtered using a Milli-Q system (EMD Millipore; Billerica, MA) was used for preparing buffers. Porcine skin sourced from a Yucatan miniature pig was purchased from Sinclair Bio Resources, LLC. (Auxvasse, MO). Human skin was obtained from NCI Cooperative Human Tissue Network (CHTN) skin repository (Charlottesville, VA).

In Vitro Permeation Test (IVPT)

Both porcine and human skin were dermatomed to a thickness in the range of 250 ± 50 μm, retaining the entire stratum corneum (SC), viable epidermis, and part of the dermis. The dermatomed skin was stored at −20°C until the day of the experiment. The skin was thawed at room temperature and cut into square-shaped pieces, 4.84 cm^2 in area. Each piece of skin was positioned between the donor and receiver chamber such that the dermis-side faced the receiver chamber of the diffusion cell. The barrier integrity of the skin was checked by recording transepidermal water loss (TEWL) using a cyberDerm RG-1 open chamber evaporimeter (cyberDERM, Inc.; Broomall, PA) prior to formulation application. Any skin piece with a reading higher than 15.0 $g/m^2/h$, indicating compromised barrier integrity, was replaced.

IVPT was performed using PermeGear flow-through In-Line diffusion cells (Hellertown, PA). All preliminary experiments were done using dermatomed skin obtained from a single Yucatan miniature pig donor in a pilot study. For the pivotal studies, dermatomed human skin from three donors was used for each treatment group. Donor 1a, 1b, 1c, 2, and 3 indicate different human skin donors. Skin samples obtained from donors 2 and 3 were large enough to be used for all four formulations. The first treatment group was the baseline temperature group where skin temperature was maintained at 32 ± 1°C to mimic normal baseline skin temperature. The second treatment group was maintained at 42 ± 1°C to mimic elevated skin temperature conditions attained due to an external heat source since skin temperatures have been reported to reach around 40–43°C upon exposure to common sources of heat (8–12). Three to four replicates per donor were used for each treatment group. Each diffusion cell had a permeation area of 0.95 cm^2. Dermatomed skin was cut, positioned between the donor and receiver chamber of the diffusion cell, and held in place by a membrane support. For the patch, a 0.97 cm^2 circular disc of the patch was applied to the permeation area of the diffusion cell. For the diclofenac sodium products (Table I), clinically relevant doses of 2% solution (5 mg/cm^2), 1% gel (10 mg/cm^2), and 3% gel (20 mg/cm^2) were applied to the skin. Isotonic potassium phosphate buffer solution at pH 7.4 ± 1.0 was used as the receiver solution maintained at 37°C in a water bath. Receptor solution was collected every two or three hours for 12 h and analyzed using a validated high performance liquid chromatography (HPLC) method. In order to prevent the patch from lifting, a 4.84 cm^2 piece of polypropylene knitted mesh (0.15 mm monofilament, 3.0 × 2.8 mm pores, 47 GSM; SurgicalMesh™ Division of Textile Development Associates, Inc.; Brookfield, CT) was applied on top of the patch disc and held in place by the donor chamber. The three semisolid formulations were dosed on the skin using a positive displacement pipette; then distributed over the permeation area of the skin using an inverted HPLC vial.

HPLC Analysis of IVPT Samples

The HPLC system consisted of a Waters® Alliance e2695 separations module and a Waters® 2489 dual-wavelength absorbance detector with Waters Empower™ software (Milford, MA). Samples were injected by an autosampler onto an Agilent Zorbax® 300SB-C8 column (3.5 μm, 4.6 × 150 mm) with Phenomenex SecurityGuard™ C8 cartridge (5 μm, 4 × 3.0 mm). Mobile phase consisting of methanol and 20 mM potassium phosphate buffer in 65:35 (*v*/v) ratio was set at a flow rate of 1 mL/min to elute diclofenac at 4.1 min. The maximum wavelength for UV detection of diclofenac was set at 280 nm. Receiver solution was diluted with methanol in a 1:1 (v/v) ratio. The diluted sample (40 μL) was injected onto the column. The concentration of the calibration standard samples ranged from 0.025 to 10 μg/mL. The method was precise with intra-day and inter-day variation less than 6% and with accuracy between 98 to 107% for all quality control (QC) samples and for the lower limit of quantification (LLOQ).

IVPT samples obtained from the two gel products were subjected to liquid-liquid extraction (LLE) due to the low concentration of diclofenac in the samples (below 0.025 μg/mL). For LLE, 1 mL of receiver solution was spiked with 100 μL of 1 μg/mL flufenamic acid as an internal standard. Phosphoric acid (0.1 M) was used to acidify the samples. Diclofenac was extracted into 3 mL of ethyl acetate after shaking for 20 min on a high-speed shaker. The mixture was centrifuged for 10 min at 1341×g. The upper organic layer (2.5 mL) was transferred into a clean microcentrifuge tube and evaporated under a light stream of nitrogen at 50°C. The residue was reconstituted with 100 μL of mobile phase and 40 μL was injected onto the HPLC column for quantification. The same HPLC conditions described above were utilized to analyze samples prepared using the LLE procedure. The

concentration of the calibration curve used for these samples ranged from 0.001 to 3 μg/mL. The method was precise with intra-day and inter-day variation less than 7% and with accuracy between 93 to 102% for all quality control (QC) samples and for the lower limit of quantification (LLOQ).

Statistical Analysis

Differences in the mean flux values and cumulative amounts were compared using student's t test and one-way ANOVA followed by Tukey's post-hoc tests. (GraphPad Prism® software version 5.0, La Jolla, CA). Statistical significance was declared at $p < 0.05$.

RESULTS

IVPT on Porcine Skin

Porcine skin is widely used as a surrogate for human skin in diffusion studies due to its structural similarity to human skin and its availability in larger quantities. As part of a pilot study, IVPT was performed on porcine skin obtained from a single donor for the four diclofenac topical products. Flux profiles for all four diclofenac products at normal and elevated temperature are shown in Fig. 1. Pseudo-zero order drug delivery from the patch was seen at baseline and elevated skin temperatures over 12 h. A decrease in lagtime was observed at the elevated temperature for the patch and solution. Heat-induced enhancement in J_{max} and cumulative amount of drug permeation were calculated by dividing the value obtained at an elevated skin temperature of 42°C by the corresponding value at a skin temperature of 32°C. Table II summarizes the calculated heat induced enhancement in flux and cumulative amount permeated, and the corresponding p values obtained from the comparison of values at the two temperatures. The most significant increase in J_{max} was seen for the 1% gel followed by the solution, patch, and 3% gel in descending order of significance based on p values. A similar order of ranking for the four products was seen in cumulative amounts of drug permeated.

IVPT on Human Skin

Pivotal studies using human skin confirmed heat-induced enhancement in drug permeation for the four products. Human skin from three different donors was used to perform IVPT on four diclofenac topical products. Mean flux profiles for the three donors for the four products are shown in Fig. 2. The patch showed a sustained increase in flux at the elevated skin temperature throughout the 12 h of wear duration accompanied by a decrease in lag time (Fig. 2). All four diclofenac formulations showed a decrease in the time required to reach J_{max} at elevated

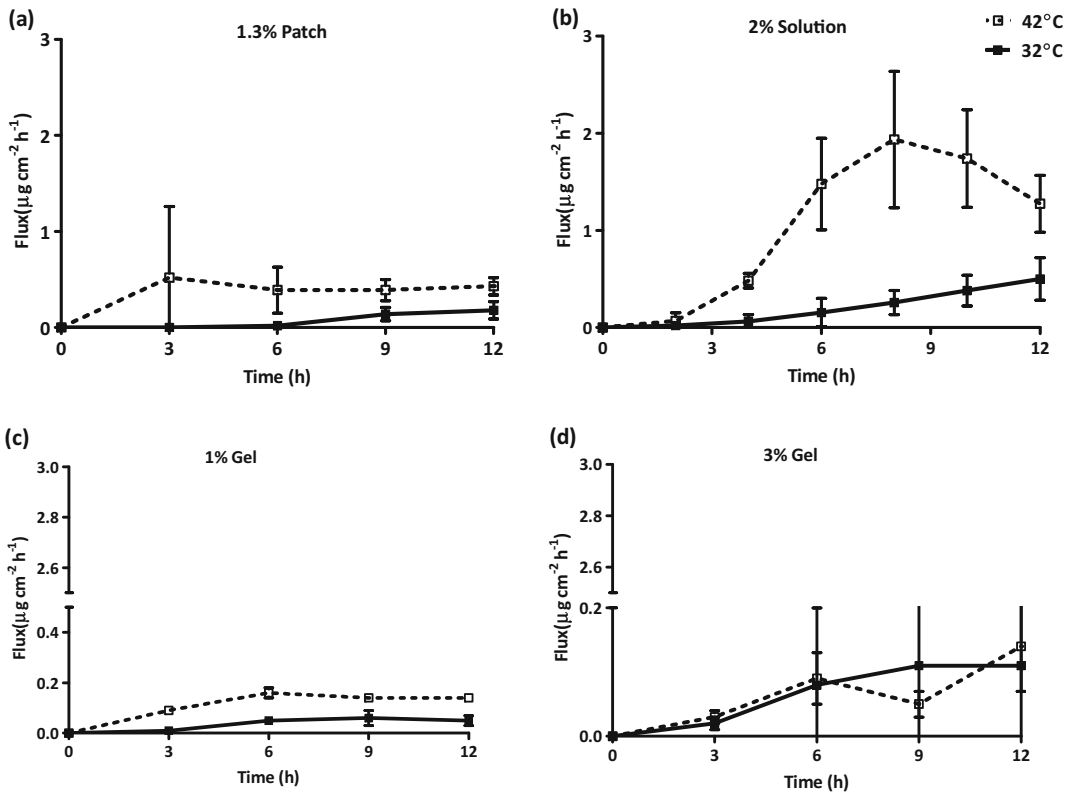

Fig. 1 Flux profile from porcine skin for 1.3% patch (**a**), 2% solution (**b**), 1% gel (**c**), and 3% gel (**d**). (mean ± SD) (n = 1 donor, 3–4 replicates/donor).

Table II Summary of Heat Enhancement on Porcine Skin ($n = 1$ donor). p values were Obtained Using Unpaired t-test

Formulation	Heat enhancement ratio (Heat/No Heat)		p value (Heat vs. No Heat)	
	J_{max}	Cum. Amt.	J_{max}	Cum. Amt.
1.3% Patch	2.3	5.0	0.034	0.104
2% Solution	4.0	5.0	0.006	0.002
1% Gel	2.6	3.0	0.001	<0.001
3% Gel	1.0	0.87	0.961	0.883

temperature (Fig. 2). At elevated skin temperature, all three semisolid formulations showed an initial rapid rise in flux followed by a slow decline, compared to their respective baseline profiles. Table III summarizes the calculated heat induced enhancement in flux and cumulative amount permeated obtained from the three donors for each product and the corresponding p values obtained from the comparison of values at the two temperatures for each individual donor per product, as well as the mean values obtained from the three donors for each product. The most significant increase in J_{max} was seen for the solution, followed by the patch and gels in descending order of significance based on p values. A similar order of ranking for the four products was seen in the cumulative amount of drug permeated. These results are consistent with the porcine skin data for three products except 1% gel. One percent gel showed greater differences at elevated temperature in flux and cumulative amount permeated on porcine skin as compared to human skin.

Figure 3a, c compare the maximum flux and cumulative amount values at 12 h for the four diclofenac products at baseline skin temperature of 32°C. Diclofenac solution (2%) showed a much larger flux and cumulative amount of drug permeation compared to patch and gels. Flux and cumulative amount values were not significantly different for the latter three products. Results for the three semisolid products were consistent with the observation on porcine skin by Cordery et al.; which attributes the increased flux of solution to its ability to deliver more drug both into and through SC compared to the gels. This inference was made based on tape-stripping data from healthy human volunteers showing a greater amount of drug in the SC at the end of the uptake period from solution compared to gels. This distinct behavior of the solution may be attributed to the presence of dimethyl sulfoxide (DMSO) in its composition (21).

Figure 3b, d compare the maximum flux values for the four diclofenac products at an elevated skin temperature of 42°C. Diclofenac solution (2%) showed a much larger flux compared to patch and gels. Flux values were not significantly different for the latter three products. These observations were similar to that at baseline skin temperature of 32°C. These differences in permeation seen among the four products at baseline and elevated temperature cannot be justified by the drug load in the applied dose. If one were to dose normalize across the three semisolid formulations, the inference would still remain the same. Hence it can be said that the inherent differences in formulation design and excipient composition of the products have an impact on drug permeation.

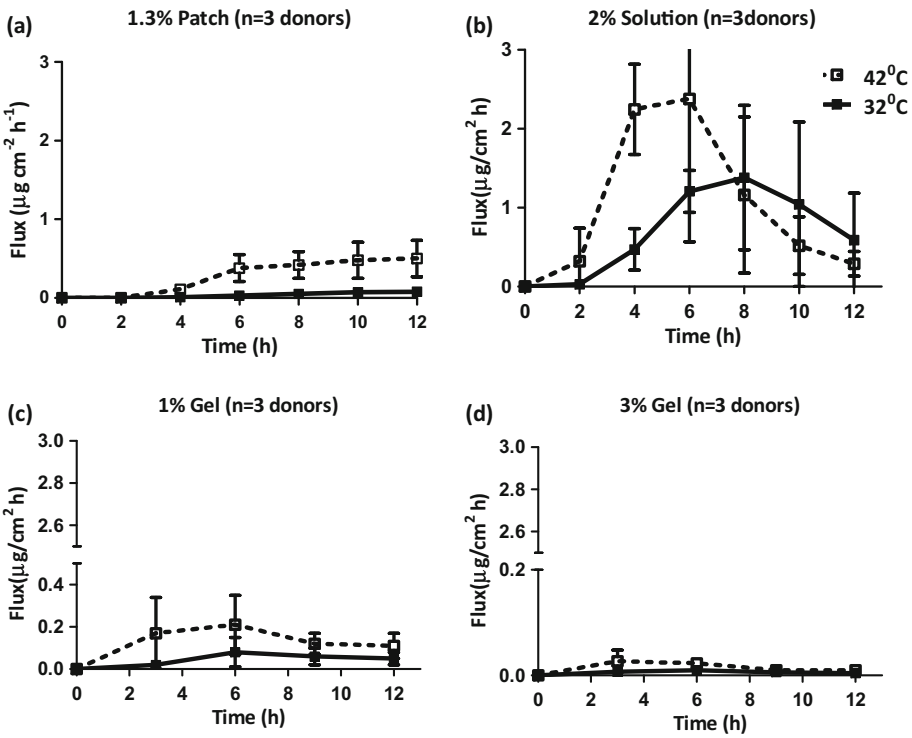

Fig. 2 Flux profile from human skin for 1.3% patch (**a**), 2% solution (**b**), 1% gel (**c**), and 3% gel (**d**). (mean ± SD) ($n = 3$ donors, 3–4 replicates/donor).

Table III Summary of Heat Enhancement on Human Skin ($n = 3$ donors). p values were Obtained Using Paired t-test for Three Donors

Formulation	Heat enhancement ratio (Heat/No Heat)		p value (Heat vs. No Heat)	
	J_{max}	Cum. Amt.	J_{max}	Cum. Amt.
1.3% Patch	6.9 ± 2.5	10.1 ± 6.0	0.079	0.067
[a]2% Solution	7.0 ± 6.4	7.1 ± 6.0	0.043	0.048
1% Gel	3.7 ± 3.0	3.9 ± 2.6	0.097	0.127
3% Gel	4.5 ± 3.5	3.5 ± 2.5	0.123	0.115

[a] Heat enhancement in J_{max} and Cum. Amt. at 4 h was calculated

DISCUSSION

Based on the physicochemical classification of drug molecules, most drugs used for topical application are either moderately or highly lipophilic weak bases. Diclofenac is representative of the weak acid drug class that is highly lipophilic with a log $P > 3$. Highly lipophilic drugs have a greater affinity for the lipophilic stratum corneum and may be slow permeating and cleared slowly from the skin (22–24). The pKa values of weak acids and bases along with the pH of the surrounding vehicle and skin layers govern the ionization profile of the molecule. Diclofenac was chosen as a model drug to represent the class of slow permeating, weakly acidic drugs. The IVPT performed demonstrated the effect of elevated skin temperature on drug permeation from four different topical diclofenac formulations, each containing different concentrations of the same API and different inactive ingredients. Comparison of flux profiles among such distinctly different products can provide us with a better mechanistic understanding of the influence of heat exposure on topically applied formulations. Unlike patches, topically applied semisolid formulations have not been widely evaluated for their ability to provide enhanced drug permeation under the influence of heat exposure. The amount of diclofenac/cm^2 contained in the clinically relevant dosing and inactive ingredients in the four products are specified in Table I. At clinically relevant dosing/cm^2, the patch has the highest drug load followed by 3% gel. The solution and 1% gel have the lowest applied drug load among the four products. Despite the lower drug load applied, the highest flux and cumulative amount of permeation levels were observed for the solution for baseline and the elevated skin temperature of 42°C (Fig. 2). This distinct behavior of diclofenac solution was also observed by Cordery

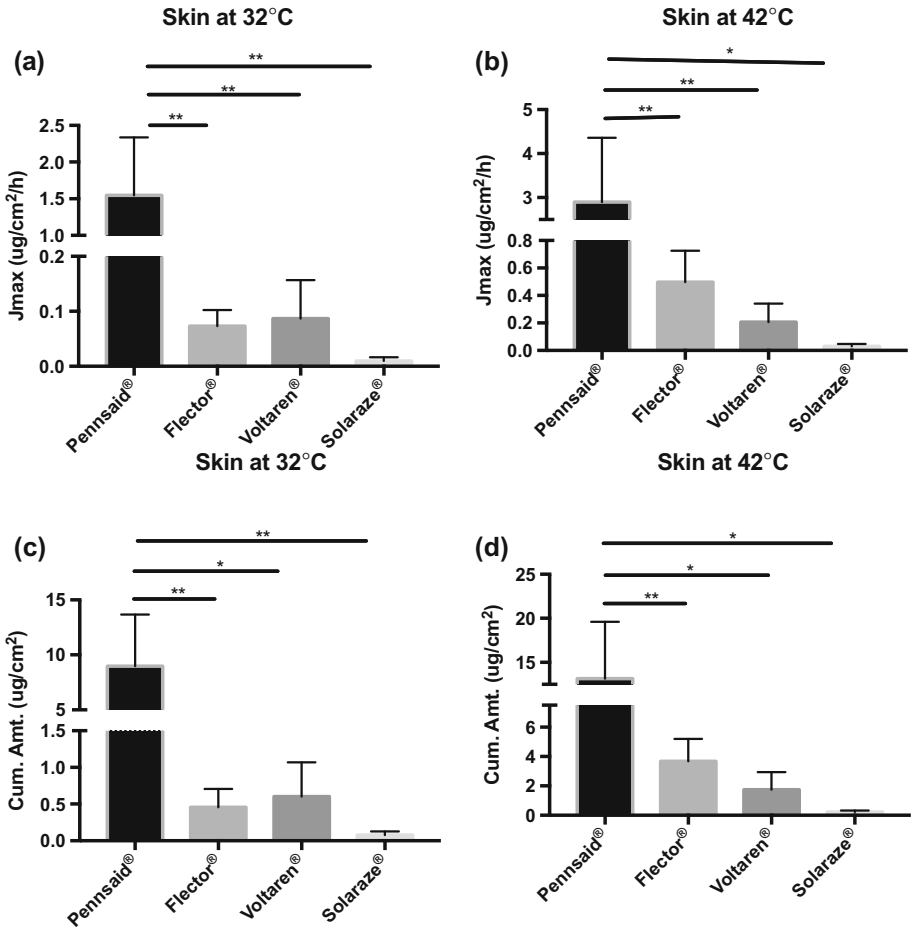

Fig. 3 J_{max} from human skin donors at skin temperature 32°C (**a**) and 42°C (**b**). Cumulative Amount at 12 h from human skin donors at skin temperature 32°C (**c**) and 42°C (**d**). (* $p \leq 0.05$; ** $p \leq 0.01$) (mean + SD) ($n = 3$ donors, 3–4 replicates/donor).

et al. and may be attributed to the high percentage (>40% *w*/w) of penetration enhancer DMSO present in the formulation (21). DMSO is known to be quickly absorbed into the SC and alters the protein and lipid structure. It possesses outstanding dissolution power which enables it to generate solvent filled spaces in the SC thereby improving the solubility of drugs (25). The patch and gels did not show statistically significant differences in penetration amounts and J_{max} values when compared to each other at baseline and elevated temperatures.

Continuous heat application enables evaluation of the worst case scenario in the case of prolonged heat exposure. The calculated flux enhancement represents the possible enhancement in plasma levels of the drug *in vivo* (Tables II and III). Many topical drugs, such as diclofenac, are not expected to reach high systemic levels. However, a spike in systemic levels of diclofenac may still pose a potential safety concern in a susceptible patient population predisposed to any CV adverse effect caused by elevated systemic levels of the drug (20). Systemic toxicity from increased percutaneous absorption of lidocaine from a topical formulation due to compromised skin barrier integrity has been reported to cause adverse reactions in patients (26).

Unlike patches, semisolids are not self-contained but are left open to air post-application. Evaporation of volatile excipients post-application can alter the composition and performance of these topically applied formulations. Flynn et al. have shown that evaporation and permeation of solvents can result in a saturated solution of the drug followed by precipitation of the drug. The initial evaporation and saturation of solution accelerate drug delivery, but this advantage is lost once the drug precipitates. The resultant thermodynamic activity of the drug drives the kinetics of topical drug delivery (27). These processes are expected to occur at a faster rate at elevated skin temperature. Volatile solvents like ethanol and water evaporate very quickly after application, after which drug is in pure non-volatile solvents like propylene glycol and polyethylene glycol which can permeate and evaporate after topical application, albeit very slowly (28). This can affect drug permeation at the later time points. In the pivotal study, the semisolid products under heat influence showed a rapid rise in flux to reach J_{max} earlier than the respective baseline flux profile. This rapid rise in flux is followed by a steady decline until a plateau is reached resulting in a dome-shaped flux profile curve. The shape is steeper for the solution compared to the two gels. In the early stage, when the vehicle (solution or gel) is in its essentially native form, the increased temperature can cause decreased vehicle viscosity, increased drug solubility, and rapid evaporation of volatile solvents. All these events result in increased thermodynamic activity of drug resulting in increased permeation. Temperature-induced decrease in vehicle viscosity has previously been suggested as the reason for increased release of acyclovir from Zovirax® cream (16). The continued heat application will eventually exhaust the solvents and consequently cause the drug to precipitate or crystallize, decreasing the drug delivery and permeation rate, regardless of the influence of clinically-relevant elevated heat. This may impact the efficacy of the solution since drug permeation from the solution under the influence of heat application decreases compared to the levels in the absence of heat after 8 h.

In the pivotal studies conducted using three human skin donors, the most significant enhancement in flux and cumulative amount of drug permeation was seen for diclofenac solution 2%. As mentioned earlier, this solution contains >40% *w*/w DMSO which is a well-known penetration enhancer. The ability of DMSO to promote drug partitioning into the SC results in a greater amount of drug (21). The low viscosity of the solution in combination with the permeation enhancing effect of DMSO results in a more significant increase in drug permeation at elevated temperature compared to the patch and gels.

The patch contains 1.3% of diclofenac epolamine in a polymeric hydrogel to compose a matrix type topical patch in contrast to the sodium salt in the semisolid formulations. The epolamine salt of diclofenac has improved solubility in both aqueous as well as organic solvents and demonstrated surfactant properties resulting in improved skin permeation compared to the sodium salt of diclofenac (29). The large reservoir of diclofenac (highest applied drug load compared to the three semisolid formulations) and the closed environment provided by the backing membrane is designed to maintain pseudo-zero order absorption kinetics. This pseudo-zero order absorption kinetics is not disrupted even under the influence of heat throughout the 12 h wear duration. Both porcine and human skin showed similar absorption kinetics. The backing membrane results in a closed environment of the patch, unlike that of semisolid formulations, which prevents any drastic changes in the physical nature of the patch formulation. At the higher skin temperature, the flux showed a rapid rise to reach steady-state flux levels more quickly than compared to the baseline profile resulting in a decreased lag time. Such increased *in vitro* permeation at 42°C has been reported for fentanyl patches (7). Despite the improved permeation of epolamine salt, the closed environment of the patch and lack of penetration enhancers prevent any drastic enhancement in drug permeation at elevated skin temperature.

When comparing heat induced enhancement results for porcine and human skin for three products, the 3% gel showed greater differences at elevated temperature in flux and cumulative amount permeated on human skin compared to porcine skin while 1% gel showed greater differences at elevated temperature in flux and cumulative amount permeated on porcine skin as compared to human skin. This could be due to the slow permeating nature of diclofenac which imparts variability to the drug permeation in different donors. In addition, the unprotected and unoccluded environment of

semisolids can impart additional variability. Porcine skin is known to be similar to human skin, but freeze thaw conditions may add to the variability (30,31). However, it is still fair to say that screening some formulations in porcine skin for heat effect in the absence of readily available human skin may be a reasonable study design. Both gels showed the least significant heat induced enhancement in flux and cumulative amount of drug permeated in human skin. The higher gel vehicle viscosities compared to the solution and lack of the large percentage of the potent DMSO permeation enhancer does not support a significant increase in drug permeation at elevated temperature. The 3% gel formulation contains 2.5% sodium hyaluronate (HA) which has been shown to enhance partitioning, retention, and localization of diclofenac in the epidermis. This results in the formation of a depot of drug in the epidermis and minimizes percutaneous absorption of diclofenac (32). The presence of HA, which limits drug permeation, could possibly help to prevent substantial increases in flux at elevated skin temperature. It is also interesting to note that despite the higher application dose/cm^2 of the 1% and 3% gels, both products provided an overall similar heat enhancement effect, as compared to the smallest formulation dose (albeit same diclofenac application/cm^2 as the 1% gel) of the 2% solution which provided the most significant heat enhancement effect. If drug load alone was predictive of magnitude of heat-induced enhancement in permeation, one might have expected that 10 mg/cm^2 of a 1% gel would have a less significant heat-enhanced absorption effect than 20 mg/cm^2 of a 3% gel, two-fold more gel excipients and six-fold more amount of drug applied/cm^2. These observations further confirm the importance of formulation components and their respective concentrations on the potential for significant heat-enhanced dermal absorption.

Taken together, these results demonstrate that application of topical diclofenac products in conjunction with external heat application can result in increased drug permeation through the skin and hence could increase systemic drug levels. According to the National Drug Monograph, an oral 50 mg tablet can result in a C_{max} of 1500–1600 ng/mL which is approximately 20-fold higher than the levels that the solution can potentially reach under the influence of heat application at clinically relevant dosing. The levels that can be potentially reached by the other three products under the influence of heat will be even lower. Hence, it can be concluded that the four diclofenac topical formulations studied in this paper are not expected to reach concerning systemic levels of drug even under the worst case scenario of heat application. However, increased systemic levels caused by exposure to heat on transdermal systems containing drugs with a narrow therapeutic window, unlike diclofenac, can be a safety concern. Among the four diclofenac products considered in this paper, only Voltaren® gel has been studied with heat application on human subjects as described in its package insert. In this study, moderate heat was applied for 15 min before application of formulation and no clinically relevant difference in systemic absorption was reported (17). Increased permeation of diclofenac in human subjects has been reported in a previous study when a heating pad was placed on top of diclofenac plasters for 4 h (33).

CONCLUSIONS

The results demonstrated that heat application in conjunction with topically applied formulations can result in increases in flux values. Events in everyday life that can result in an elevated skin temperature have the potential to alter the pharmacokinetic profile of topical products. Topical products usually are not expected to reach high systemic levels, but it has not been well characterized whether elevated plasma levels could be a result of unintentional heat exposure. Our results show that predicted increases in drug levels resulting from heat application may not exceed drug exposure resulting from a therapeutic oral dose of diclofenac. Hence, under heat exposure, diclofenac topical formulations are not expected to reach systemic levels that are of concern. The relatively large drug reservoir and closed environment of the patch design may enable it to maintain a sustained increase in drug permeation throughout the duration of heat application. In the case of the semisolid formulations, a transient increase in drug permeation is seen due to the nature of the semisolid formulation. At elevated temperature, the altered viscosity and accelerated vehicle evaporation would affect the thermodynamic activity of the drug, which results in altered permeation. Formulation design and excipients can influence the extent or significance of heat-induced enhancement in drug permeation.

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