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Research paper

Influence of Transcutol® CG on the skin accumulation and transdermal permeation of ultraviolet absorbers

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Abstract

The objective of this study was to determine the influence of Transcutol® CG concentration on the transdermal permeation and skin accumulation of two ultraviolet (UV) absorbers, 2-hydroxy-4-methoxybenzophenone (oxybenzone) and 2-octyl-4-methoxycinnamate (cinnamate). The concentration of the UV absorber was held constant at 6% (w/w) for all vehicle systems while the concentration of Transcutol® CG was varied from 0 to 50% (w/w). Data showed that both UV absorbers exhibited increases in skin accumulation with increasing concentrations of Transcutol CG. Skin accumulation of oxybenzone was significantly ($P < 0.05$) greater than that of cinnamate for all formulations investigated. Oxybenzone skin accumulation ranged from 22.9 ± 2.8 μg/mg (0% Transcutol® CG) to 80.8 ± 27.2 μg/mg (50% Transcutol CG). Cinnamate skin accumulation ranged from 9.0 ± 0.9 μg/mg to 39.8 ± 12.2 μg/mg at 0 and 50% Transcutol CG, respectively. No significant differences were found in the transdermal permeation of oxybenzone or cinnamate for any of the formulations tested. The results of this study demonstrate that the inclusion of Transcutol CG in sunscreen formulations increases the skin accumulation of the UV absorbers oxybenzone and cinnamate without a concomitant increase in transdermal permeation.

Keywords: Skin accumulation; Transcutol; Sunscreen; Ultraviolet radiation; Transdermal permeation

1. Introduction

Skin cancer is the most common type of cancer in the world today, accounting for nearly half of all reported cancer incidences [1]. In recent years, the use of topical sunscreen products has been advocated as the best prevention of skin cancer. These products are chemical agents in the form of solutions, gels, creams, or ointments that diminish the deleterious effects of exposure to ultraviolet (UV) radiation on skin. Sunscreen products provide their photoprotection by the active ingredient(s) absorbing, reflecting, and/or scattering solar UV radiation [2]. For sunscreens to be effective, the UV absorbers must remain in the outermost regions of the skin [3]. An ideal sunscreen product should exhibit high skin accumulation of UV absorbers with minimal permeation to the systemic circulation.

The rate of transdermal permeation of a compound is dependent on the properties of the compound, the integrity of the skin, and the physico–chemical characteristics of the vehicle in which the compound is dissolved or dispersed [4,5]. Although many scientists have focused their research efforts towards maximizing transdermal absorption of drugs [6,7], permeation of UV absorbers is undesirable. Once the UV absorbers have permeated into the systemic circulation, photoprotection is lost and the skin is susceptible to damage from the sun. While a number of commonly used UV absorbers have been shown to be rapidly absorbed into the systemic circulation [8], little research has been performed to investigate mechanisms to increase the skin accumulation of these compounds.

Transcutol® CG (diethylene glycol monoethyl ether) is a hydroscopic liquid that is freely miscible with both polar and non-polar solvents. Transcutol has been recognized as a potential transdermal permeation enhancer due to its non-toxicity, biocompatibility with skin, and excellent solubilizing properties. However, Transcutol has also been reported to increase the skin accumulation of topically applied compounds without a concomitant increase in transdermal permeation [9]. The objective of this study was to determine the influence of Transcutol® CG concentration in sunscreen formulations on the transdermal permeation and skin accumulation of the UV absorbers 2-hydroxy-4-methoxybenzophenone (oxybenzone) and 2-octyl-4-methoxycinnamate (cinnamate).

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2. Materials and methods

2.1. Materials

2-Hydroxy-4-methoxybenzophenone (oxybenzone) and 2-octyl-4-methoxycinnamate (cinnamate) were purchased from Aldrich (Milwaukee, WI). These compounds differ in their physico–chemical properties and UV wavelengths absorbed. Isopropyl myristate (IPM) was purchased from Spectrum Chemical (New Brunswick, NJ). Aqueous formaldehyde solution (37%) and Brij 58 (polyoxyethylene (20) cetyl ether) were purchased from Aldrich (Milwaukee, WI). Transcutol® CG was kindly provided by Gattefossé Corporation (Westwood, NJ). All solvents used in this study were High Performance Liquid Chromatography (HPLC) grade and were purchased from JT Baker (Phillipsburg, NJ). Chemicals were used as received.

2.2. Preparation of sunscreen formulations

The concentrations of the UV absorbers (FDA mandated maximum concentration of 6%) [10] were held constant for all vehicle systems. The solvent system consisted of Transcutol® CG in concentrations of 0, 10, 17.5, 25, or 50% (w/w), with IPM comprising the remainder of the solvent. Ten grams of each formulation were prepared prior to experimentation. The UV absorber (0.6 g) was accurately weighed, dissolved in IPM, then Transcutol® CG was added. The UV absorber/solvent mixtures were stirred using a magnetic stir bar until a clear solution was obtained.

2.3. In vitro transdermal permeation

Eight-week-old male SKH-1 hairless mice were obtained from Charles Rivers Laboratories (Wilmington, MA). The animal protocol was approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee. Animals were sacrificed by CO_2 asphyxiation and full-thickness abdominal and dorsal skin was excised. Any extraneous subcutaneous fat was removed from the dermal surface. The skin samples were stored at −80°C (Revco Scientific, Asheville, NC) until utilized. Research involving a variety of skin types, including human, cattle, and nude rat, has demonstrated that freezing skin prior to experimentation does not alter the transport kinetics of skin [11–14]. At the time of experimentation, skin samples were slowly thawed, cut into four pieces, and mounted on modified Franz diffusion cells (Permegear, Riegelsville, PA). Each diffusion cell (donor surface area 0.64 cm^2; receptor volume 5.1 ml) contained isotonic phosphate buffer solution (pH 7.2), 0.1% v/v 37% aqueous formaldehyde as a preservative and 0.5% w/v Brij 58 as a solubilizer. The receptor fluid was maintained at 37 ± 0.5°C and continuously stirred at 600 rpm using magnetic stirring bars. The solubility of oxybenzone and cinnamate at 37°C was 240 mg/l and 388 mg/l, respectively and sink conditions were maintained over the course of the experiment. Following a 1-h hydration period, 200 μl of sunscreen formulation was spread uniformly over each skin. Samples (300 μl) of the receptor phase were withdrawn at specified time points over 4 h and immediately replaced with fresh buffer. Analysis of samples was corrected for previous UV absorber removed.

2.4. In vitro skin accumulation

The skin samples were removed from the receptor cells 4 h after application of the sunscreens. The skin samples were washed three times in 100 ml of methanol for a total of 15 s to remove residual formulation from the surface of the skin. Following room temperature drying, each skin was weighed, cut into small pieces, placed in 2 ml of methanol, and homogenized using a tissue homogenizer (Biospec Products, Racine, WI). The homogenate was then centrifuged for 5 min at 13,000 rpm using a Fisher benchtop centrifuge (Pittsburgh, PA). Following centrifugation, 1 ml samples of the supernatant were removed and stored at −80°C until analyzed.

2.5. Analytical method

Analysis of samples was performed using HPLC. The liquid chromatograph consisted of a binary pump solvent delivery system (Model P1500, Thermoseparations Products, Riviera Beach, FL), a 50 μl injection loop autosampler (Model AS 1000, Thermoseparations Products), and a variable-wavelength ultraviolet light absorbance detector (Model UV 1000, Thermoseparations Products). The analytical column was a 5 μm pore size, 4.6 × 150 mm Microsorb-MV C_{18} column (Rainin Instrument Company, Woburn, MA) with a guard column of the same material. The system was controlled and integrated by a personal computer using chromatography management software (PC 1000, Thermoseparations Products). The detection wavelength was 288 nm for both UV absorbers. The mobile phase was methanol:water containing glacial acetic acid at a concentration of 0.01% (v/v) (83:17 for oxybenzone and 90:10 for cinnamate) and the flow rate was 1 ml/min [8]. Retention times for oxybenzone and cinnamate were approximately 6 and 9 min, respectively.

2.6. Data analysis

Transdermal permeation was determined by analyzing each receptor compartment sample for UV absorber and is reported as μg/cm^2. Skin accumulation was calculated by dividing the amount of UV absorber remaining in the skin by the weight of the skin sample. Skin accumulation is reported as μg of UV absorber/mg of skin. The mean and standard deviation of these parameters were calculated. Statistical analysis was carried out using SigmaStat (SPSS Inc., Chicago, IL). For statistical comparison, a one-way ANOVA was employed. A Tukey posttest was then used

to determine differences between treatment groups. A $P < 0.05$ was considered statistically significant.

3. Results

The current study examined the effects of Transcutol® CG on the transdermal permeation and skin accumulation of two commonly used UV absorbers, 2-hydroxy-4-methoxybenzophenone (oxybenzone) and 2-octyl-4-methoxycinnamate (cinnamate). Increasing the concentration of Transcutol CG in the solvent system resulted in significant increases in skin accumulation for both UV absorbers, as shown in Fig. 1. For oxybenzone, a statistically significant ($P < 0.05$) increase in skin accumulation was seen between control (22.9 ± 2.2 μg/mg) and 17.5% Transcutol CG (65.5 ± 22.4 μg/mg). The highest skin accumulation of oxybenzone was found with 50% Transcutol CG (80.8 ± 27.2 μg/mg), although this value was not significantly higher than the formulation containing 17.5% Transcutol ($P = 0.449$). The percentages of applied oxybenzone that accumulated in the skin ranged from 9.0% from the control formulation to 35.0% for the 50% Transcutol formulation. Similar results were found with cinnamate, where a significant increase in skin accumulation was found between control (8.9 ± 0.9 μg/mg) and 17.5% Transcutol CG (23.0 ± 8.8 μg/mg). As with oxybenzone, the highest skin accumulation of cinnamate was found with the 50% Transcutol CG formulation (39.8 ± 12.1 μg/mg). Percentages of applied cinnamate that accumulated in the skin ranged from 3.0% from control to 18.5% in the 50% Transcutol formulation.

Data from the current study showed that inclusion of Transcutol in the UV absorber formulations did not significantly increase the transdermal permeation of oxybenzone or cinnamate (Figs. 2 and 3) at any time point investigated (0–4 h). Permeation from the control formulation (0% Transcutol) was low for both oxybenzone and cinnamate. The highest permeation of each UV absorber was found for the formulation containing 10% Transcutol. Increasing the concentration of Transcutol CG in the formulation to 17.5 and 25% resulted in decreased transdermal permeation for both UV absorbers, while permeation of these compounds from 50% Transcutol formulations was equal to or less than control.

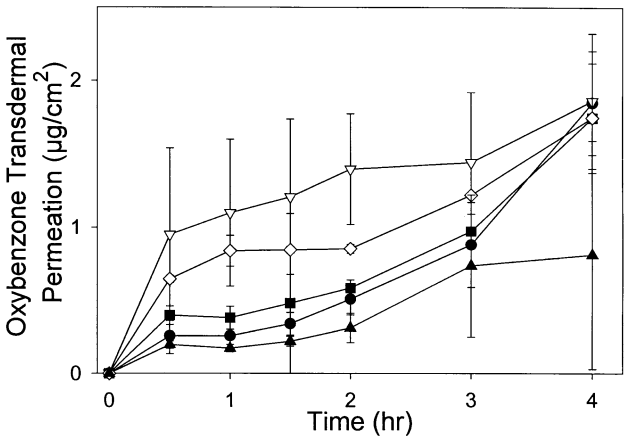

Fig. 2. Transdermal permeation of oxybenzone as a function of Transcutol® CG concentration. (●) 0% Transcutol; (▽) 10% Transcutol; (■) 17.5% Transcutol; (◇) 25% Transcutol; (▲) 50% Transcutol.

4. Discussion

Our findings are similar to those presented by Panchagnula and Ritchel, who published the first report of increased skin accumulation of drug using Transcutol. These researchers demonstrated that skin accumulation of hydrocortisone (HC) and dexamethasone (DM) from saturated Transcutol solutions increased by at least an order of magnitude over control (100% distilled water), while the overall transdermal permeation decreased. Their experiments examining differ-

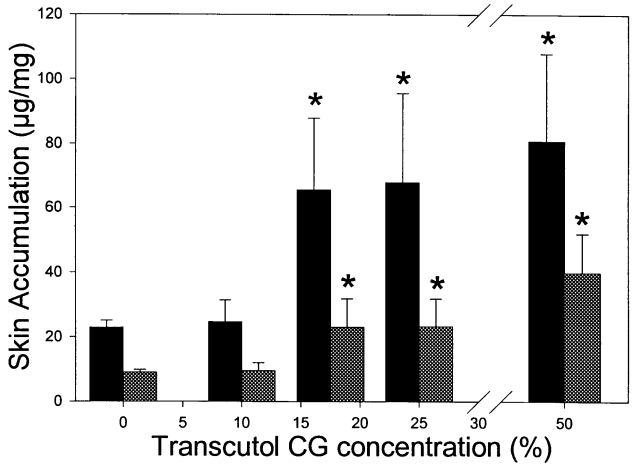

Fig. 1. Influence of Transcutol® CG concentration on skin accumulation of UV absorbers. (■) Oxybenzone, (▤) Cinnamate. (*Indicates significantly different from control [0% Transcutol]).

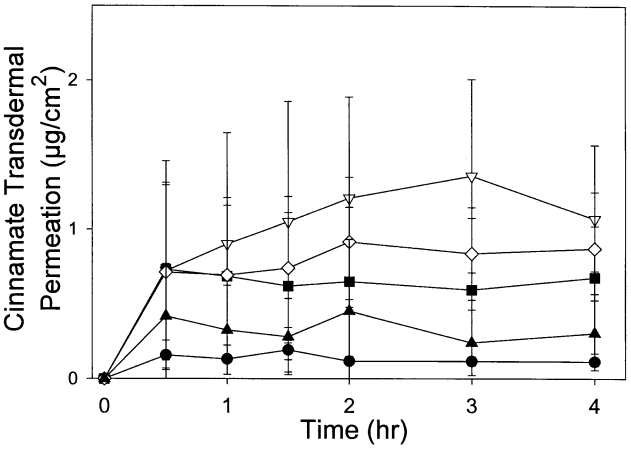

Fig. 3. Transdermal permeation of cinnamate as a function of Transcutol® CG concentration. (●) 0% Transcutol; (▽) 10% Transcutol; (□) 17.5% Transcutol; (◇) 25% Transcutol; (▲) 50% Transcutol.

ent ratios of Transcutol to water demonstrated that the skin accumulation of DM and HC increased as a function of Transcutol concentration, reaching a plateau at 50% Transcutol [15]. Additional work established that topical application of HC in a 50% Transcutol vehicle also resulted in a more localized and uniform distribution of the drug in the upper layers of the skin (i.e. stratum corneum) [9]. For UV absorbers, localization in the upper layers of skin would be expected to enhance photoprotection, as these compounds should absorb or scatter the UV radiation before reaching the deeper, more vulnerable viable epidermis and dermis.

The mechanism behind the increased skin accumulation of drugs due to the inclusion of Trasnscutol has been termed an 'intracutaneous depot' [15]. It is theorized that this depot effect is created by a swelling of stratum corneum intercellular lipids without alteration of their multiple bilayer structure. These swollen lipids then retain drugs (especially lipophilic compounds) to form the depot. The end result of this depot formation is an increased skin accumulation of drug with a simultaneous decrease in transdermal permeation. The data from the current study support the theory of an intracutaneous depot for UV absorbers. Both oxybenzone and cinnamate are lipophilic compounds that have been shown to permeate through the skin into the systemic circulation [8]. In the current study, the skin accumulation of each of the UV absorbers was significantly increased with the addition of Transcutol to the formulation without increasing transdermal permeation.

Conflicting literature exists concerning the use of Transcutol to increase the systemic delivery of topically applied medications. While permeation enhancing effects were seen for both prostaglandin [16] and theophylline [5], no permeation enhancement was noted with morphine [17]. It is possible that the permeation enhancing effects of Transcutol are concentration dependent. In the current study, the inclusion of low (10%) concentrations of Transcutol produced the highest transdermal permeation of the formulations investigated. Further increasing the concentration of Transcutol in the sunscreen formulations produced a decrease in transdermal permeation of both UV absorbers, with formulations containing 50% Transcutol exhibiting permeation values equal to or less than control.

Transcutol is thought to provide its permeation enhancing function by increasing the solubility of the applied compound. Generally, an increase in solubility results in an increase in drug permeation through the skin. However, Mura and coworkers studied Transcutol both alone and in combination with propylene glycol (PG) as a permeation enhancer for clonazepam from Carbopol gels. Despite large (200-fold) increases in clonazepam solubility in 50% Transcutol gels, the researchers found that the permeation through excised rabbit ear skin was significantly decreased, with a simultaneous increase in clonazepam skin accumulation [18]. The data from the current study is in agreement, as increasing the concentration of Transcutol up to 50% in the UV absorber vehicle system resulted in an increase in skin accumulation with no increase in skin permeation.

Interestingly, the skin accumulation of oxybenzone was significantly ($P < 0.05$) higher than that of cinnamate at all concentrations of Transcutol CG investigated despite equal amounts of UV absorbers being applied to the skin. Percutaneous absorption initially involves the release of a compound from the vehicle and then diffusive permeation through the stratified skin into the systemic circulation [19]. Drug release from the vehicle is controlled by thermodynamic factors, such as solubility, while diffusion is controlled by kinetic factors, such as diffusivity and permeability. In the present study, the solubility parameters of the UV absorbers and vehicles were calculated and are presented in Table 1. In general, large differences in the solubility parameters of a drug and its vehicle are indicative of more extensive partitioning of the drug out of the vehicle. As seen in Table 1, the differences in solubility parameters between oxybenzone and the vehicles is much larger than the differences between cinnamate and the vehicles. Thus, based on thermodynamic factors, oxybenzone would be expected to partition more readily from the vehicle.

Kinetically, the diffusion of a compound through the skin is controlled by its size and lipophilicity. Smaller compounds penetrate more rapidly into the skin, while a parabolic relationship exists between compound lipophilicity and its rate of transdermal permeation, with extremely lipophilic or hydrophilic compounds having difficulty permeating through the skin [20]. For topical application, a compound should ideally possess a log P value between 1 and 3 [21]. Oxybenzone has a smaller molecular volume than cinnamate and a log P value considered optimal for topical drug delivery, as seen in Table 1. Based on these thermodynamic and kinetic factors, oxybenzone should theoretically have both higher skin accumulation and trans-

Table 1
Physicochemical properties of UV absorbers and vehicles.

Compound	Solubility parameter $(J/cm^3)^{1/2}$[a]	Lipophilicity (log P)[b]	Molecular volume ($Å^3$)[b]
Oxybenzone	7.8	2.73	259.03
Cinnamate	11.4	4.14	350.21
Transcutol	19.2	N/A	N/A
Isopropyl myristate	18.4	N/A	N/A

[a] As calculated per Van Krevelen method [22].
[b] Calculated using a molecular modeling program (Spartan 5.0, Wavefunction, Inc. Irvine, CA).

dermal permeation than cinnamate. While the data shows that oxybenzone does indeed demonstrate significantly higher skin accumulation than cinnamate, the data also reveal no significant differences in transdermal permeation between the two compounds at any of the time points investigated. These data suggest that oxybenzone partitioned more extensively out of the vehicles into the skin only to become trapped within the swollen stratum corneum lipids. Cinnamate also demonstrated increased skin accumulation, but not to as great an extent, presumably due to lower partitioning out of the vehicle.

It can be concluded that increasing the concentration of Transcutol® CG in a mixture of isopropyl myristate results in significant increases in the skin accumulation of the UV absorbers oxybenzone and cinnamate without a concomitant increase in their transdermal permeation suggesting the formation of an intracutaneous depot. It is expected that increased skin accumulation of UV absorbers will increase substantivity of sunscreens and provide longer lasting photoprotection. Further investigations, however, are needed to correlate increased skin accumulation of UV absorbers to enhanced photoprotection.

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