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RESEARCH PAPER

Influence of Cyclodextrin Complexation on the In Vivo Photoprotective Effects of Oxybenzone

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ABSTRACT

The objective of the current study was to investigate the influence of cyclodextrin complexation on the in vivo photoprotective effects of a model ultraviolet (UV) absorber, oxybenzone, and to compare these novel sunscreens to a commercial SPF 30 sunscreen product. Aqueous-based solutions and suspensions containing 2.7 mg/mL oxybenzone and up to 20% (w/w) hydroxypropyl-β-cyclodextrin (HPCD) were prepared. The sunscreens were applied to the dorsal skin of SKH-1 hairless mice and the animals were exposed to up to two minimal erythemal doses (MEDs) of UV radiation. Control animals received no sunscreen treatment. Lipid damage, as quantified by decreases in the lipid melting temperature of the epidermis, was determined using differential scanning calorimetry immediately after UV exposure. The number of sunburn cells (SBCs) and the extent of edema were measured 24 hours postexposure. Results showed that all oxybenzone-containing formulations decreased the number of SBCs formed, diminished swelling, and reduced the physical damage to the skin structure, in comparison to control. Thus, complexation did not prevent oxybenzone from reacting with light. The 20% HPCD formulation exhibited more substantial photoprotection at UV exposures of one or two MEDs, as evidenced by the formation of fewer SBCs. The 5% HPCD formulation also provided substantial protection against epidermal lipid damage. These studies demonstrate that inclusion of HPCD in sunscreen formulations may enhance the in vivo photoprotective effects of the UV absorbers. No single HPCD-containing sunscreen, however, was found to be equivalent to a commercially available sunscreen product for all biomarkers investigated.

Key Words: Complexation; Hydroxypropyl-β-cyclodextrin; Oxybenzone; Sunscreen; Photoprotection.

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INTRODUCTION

Skin cancer is the most common type of cancer in the United States, accounting for nearly half of all reported incidences of cancer. Approximately 1,000,000 cases of nonmelanoma skin cancer are diagnosed each year. In addition, over 56,000 cases of melanoma are identified annually, resulting in more than 10,000 deaths.[1] Both melanoma and nonmelanoma skin cancers are associated with damage caused by sun exposure, and the use of sunscreens has been advocated as the best prevention of these cancers.[2] Sunscreen use also limits acute photodamage, such as sunburn, erythema, and edema. Sunscreens contain ultraviolet (UV)-absorbing compounds that filter and absorb solar radiation. To be effective, the UV absorbers must remain on the surface of the skin. Despite the extensive use of sunscreens, a number of commonly used UV absorbers, including oxybenzone, has been shown to rapidly permeate through the skin and reach the systemic circulation, leaving the skin unprotected against solar radiation.[3,4]

Preliminary studies have investigated the addition of cyclodextrins to sunscreens as a method to reduce the rate of transdermal permeation.[5] Cyclodextrins are hydrophilic, cyclic oligosaccharides that form inclusion complexes with lipophilic compounds. These compounds have been used in pharmaceutical products to increase aqueous solubility and improve chemical stability of various drugs.[6–8] Cyclodextrins have also been included in solid and semi-solid formulations.[9–12] Our preliminary studies showed that cyclodextrins complex with the UV-absorbing compound oxybenzone (commonly found in commercial sunscreens) and may create a reservoir on the skin surface.[5] The sunscreen complex was too large and hydrophilic to readily penetrate into the skin and cyclodextrin concentrations in excess of that necessary to solubilize oxybenzone produced a significant decrease in transdermal flux. The objective of the current study was to determine the in vivo photoprotective effects of oxybenzone in the presence of varying amounts of cyclodextrin and compare the extent of photoprotection provided by these novel sunscreens to a commercially available SPF 30 sunscreen product.

MATERIALS AND METHODS

2-Hydroxy-4-methoxybenzophenone (oxybenzone) and 0.8 molar substituted 2-hydroxypropyl-β-cyclodextrin (HPCD) were purchased from Aldrich (Milwaukee, WI). Trypsin 10x solution was purchased from Sigma (St. Louis, MO). Formalin was purchased from EM Science (Gibbstown, NJ). The commercial SPF 30 sunscreen (No-ad® Ultra Sun Block lotion, Solar Suncare, Miami Lakes, FL), a w/o lotion containing oxybenzone, octyl methoxycinnamate, and octyl salicylate as the active ingredients, was purchased from a local pharmacy. All chemicals were used as received. Six-week-old SKH-1 hairless mice were purchased from Charles River Laboratories (Wilmington, MA).

Preparation of Oxybenzone-Containing Sunscreen Formulations

Aqueous solutions and suspensions of oxybenzone containing up to 20% HPCD were prepared. The concentration of oxybenzone was held constant at 2.7 mg/mL for all formulations. This concentration was selected based on the solubility of the UV absorber in a 10% HPCD solution.[5] Thus, formulations containing 0% or 5% HPCD were suspensions while solutions were formed at higher HPCD concentrations. The HPCD was dissolved in distilled water and then the oxybenzone was added. The formulations were covered and stirred overnight using a magnetic stir bar.

In Vivo Ultraviolet Radiation Exposure

The hairless mouse model was used for the photoprotection studies. This model has been used extensively to investigate the histological, ultrastructural, biochemical, and immunological effects of UV radiation on skin and its relationship to photodamage and skin cancer.[13,14] The animal protocol was approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee. Prior to experimentation, four hairless mice were anesthetized with ketamine (100 mg/kg IP) and a rectangular area approximately 2.5 cm × 4 cm was marked off on the dorsal side of each animal. The HPCD/oxybenzone formulations and the commercial sunscreen were applied at 2 mg/cm^2. Control mice were left untreated. After a 15-minute drying period [as per Food and Drug Administration (FDA) guidelines on sunscreen testing], the unrestrained mice were placed in a clear plastic box, covered with a barred lid and then irradiated. Ultraviolet radiation was produced by a planar array of two UVA-340 fluorescent ultraviolet lamps (Q-panel Company, Cleveland, OH). These lamps simulate UV radiation present in sunlight from 295 nm to 365 nm and have been used previously to investigate the effectiveness of sunscreens.[15,16] Irradiance was measured using a Model

3D V2.0 Erythema UVA and UVB Intensity Meter (Solar Light Company, Philadelphia, PA) and found to be 17.8 W/m^2 UVA and 1.5 W/m^2 UVB. The minimal erythemal dose (MED) of UV radiation for the hairless mouse is approximately 140 mJ/cm^2[17] and mice were exposed to 0, 70, 140, or 280 mJ/cm^2 (equivalent to 0, 0.5, 1.0, and 2.0 MED, respectively). One MED is defined as the amount of UV radiation necessary to cause a slight reddening of the skin 24 hours after exposure. Animals treated with the commercial sunscreen product were exposed only to the highest exposure level (280 mJ/cm^2 or 2.0 MED).

In Vivo Lipid Damage

Immediately following UV exposure, two mice were sacrificed by CO_2 asphyxiation and full thickness, dorsal skin was removed by blunt dissection. The epidermis was separated from the full-thickness skin by placing the skin dermis side down on filter paper saturated with 2.5% (w/v) trypsin solution. After storage at 37° C for 4 hours, epidermal sheets were gently lifted from the skin using forceps and then covered with fresh trypsin solution and stored at 37° C for 1 hour. Trypsin was removed from the epidermal sheets with gentle rinsing using deionized water.[18] The epidermal samples were then stored at room temperature and 75% relative humidity overnight. Following storage, approximately 10-mg samples of epidermis were sealed in aluminum pans and analyzed using a 2920 Modulated Differential Scanning Calorimeter (MDSC, TA Instruments, New Castle, DE). The samples were scanned at a heating rate of 5° C/min from 10° C to 80° C with a temperature modulation of 0.759° C/min. Lipid melting temperatures were determined using TA Instruments Universal Analysis software, with the endothermic peak quantified using peak integration and a linear baseline.

In Vivo Edema

Immediately prior to irradiation, the thickness of skin folds at the back of the neck of two sedated mice was measured using a spring-loaded pocket thickness gauge (no. 7309, Mitutoyo Corporation, Kawasaki, Kanagawa, Japan). Three measurements were taken for each mouse and these data were used as the baseline. Twenty-four hours post-UV exposure, the mice were sacrificed by CO_2 asphyxiation and the skin fold thickness was immediately measured in an identical manner. Edema was calculated as the difference in skin fold thickness between the baseline and post-UV exposure data.

In Vivo Sunburn Cell Formation

Immediately following postexposure edema measurements, the UV-exposed dorsal skin of each mouse was removed and fixed in 10% formalin solution. Two nonsequential sections of the skin were removed from each mouse, mounted to a slide, and Hematoxylin and Eosin stained. The stained skin samples were subjected to microscopic examination (600 X) and the number of SBCs per linear centimeter was calculated. Counts were done on 1.5-cm sections of interfollicular epidermis.

Data Analysis

The means and standard deviations of lipid melting temperature, edema, and SBCs were determined. Statistical analysis employed a one way analysis of variance (ANOVA) and Tukey posthoc test to determine if significant differences existed in the data. A $p<0.05$ indicated significance.

RESULTS AND DISCUSSION

The two types of commercially available sunscreens include 1) organic UV absorbers that filter and absorb solar radiation and 2) inorganic, insoluble particles that absorb and scatter light. Both types of sunscreens must remain on the surface or in the outermost layers of the skin to react with light and prevent the damaging UV rays from penetrating into the dermis.[3] Sunscreens may be removed from the skin surface by swimming, toweling off, or sweating. In addition, several of the organic UV absorbers have been shown to permeate through the skin into the systemic circulation.[19,20]

An earlier study investigated cyclodextrin complexation of oxybenzone as a method to slow transdermal permeation of this organic UV absorber commonly found in commercial sunscreens.[5] Most organic sunscreens function by absorbing UV energy, which causes excitation of electrons followed by a release of the energy through conformational changes in the absorber as the electrons return to ground state.[3] A previous study conducted by Scalia and coworkers showed that cyclodextrin complexation improved the photostability of butyl-methoxydibenzoylmethane (BM-DBM), another sunscreen agent.[21] Since these UV absorbers protect the skin by reacting with light, the photoprotective effects of complexed oxybenzone were investigated in the current study.

Sunburn Cell Formation

The acute biological responses to UV radiation have been well-characterized by erythema, pain, pruritus, and the formation of sunburn cells. Sunburn cells (SBCs) are apoptotic keratinocytes that have absorbed a lethal dose of UV radiation and have been observed in humans, mice, rabbits, and guinea pigs.[22] These cells provide a quantifiable end-point of acute UV-induced damage.[23] As shown in Fig. 1, these cells have an enlarged, pyknotic nucleus and dark, vacuolated cytoplasm. Peak SBC formation occurs 24–48 hours following UV exposure, even at suberythemal doses.[24,25] The formation of SBCs has been studied extensively as an indication of acute photodamage.[23,26,27] Some research suggests that the formation of SBCs may be a molecular response to eliminate cells unable to repair UV radiation-induced damaged DNA.[23]

In the current study, the number of sunburn cells (SBCs) formed in the mouse skin as a function of UV exposure for both the control and treatment groups was determined, and the data are presented in Fig. 2. The number of SBCs present in the skin samples increased with increasing UV exposure for the control group, from 1.5 ± 0.6 at 0 MED to 35.5 ± 5.3 at 2 MED. Small increases in SBCs in the skin of the HPCD/sunscreen-treated groups compared to the unexposed group were noted at suberythemal doses of 0.5 MED, with approximately 7 SBC/cm noted in all animals. Interestingly, maximum SBC formation occurred at 1 MED for all treatment groups and higher UV exposure did not produce additional cellular damage. No statistical differences in SBC counts were found between the 0, 5, and 10% HPCD formulations at both 1 and 2 MEDs (approximately 15 SBCs/cm). Animals treated with the 20% HPCD formulation exhibited significantly lower SBCs in comparison to the other HPCD-containing formulations investigated at both 1 and 2 MEDs ($p<0.05$), with approximately 6.5 SBCs/cm noted. These data suggest that the inclusion of 20% HPCD in the formulation enhanced the photoprotective effects of oxybenzone.

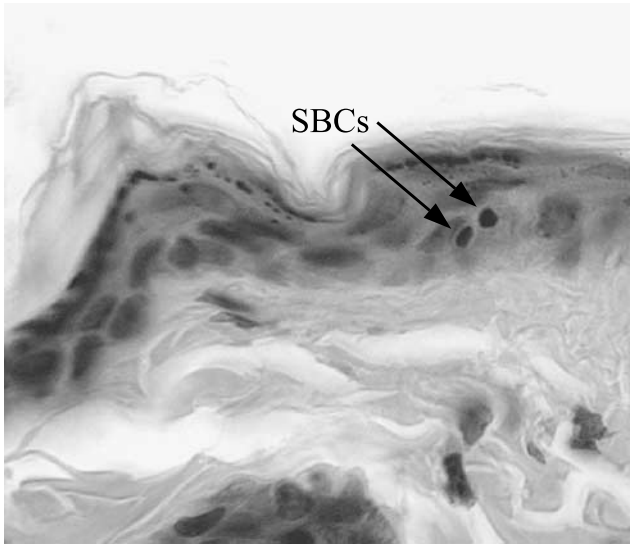

Figure 1. Microscopic photograph (600 X) depicting sunburn cells (SBCs) in UV-exposed hairless mouse skin.

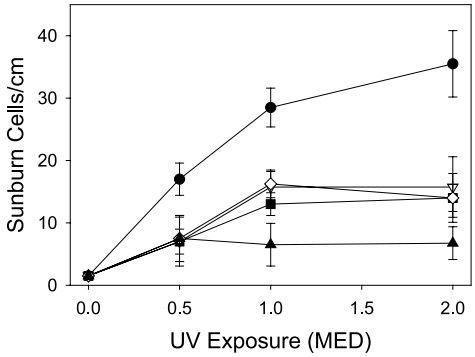

Figure 2. Influence of UV exposure on the number of sunburn cells formed as a function of HPCD in the topical formulation. ●, Control; ▽, 0% HPCD; ■, 5% HPCD; ◇, 10% HPCD; ▲, 20% HPCD.

These data may be directly related to the rate and extent of oxybenzone transdermal permeation. Our previous in vitro study showed that both flux and transdermal permeation were reduced in the 20% HPCD-oxybenzone formulation.[5] Fig. 3 depicts the theoretical equilibrium between free and complexed drug. The molecular size and hydrophilic character of the cyclodextrin-oxybenzone complex prevented any significant skin penetration from this species. The authors hypothesized that high cyclodextrin concentrations shift the theoretical equilibrium of the complexation reaction towards the complexed form, creating a reservoir of the sunscreen on the surface of the skin. Oxybenzone molecules had a greater chance of complexing with HPCD than interacting with and penetrating into the skin. The results from the current study support this hypothesis, with more of the UV absorber on the surface of the skin providing enhanced photoprotection, as evidenced by the formation of fewer SBCs.

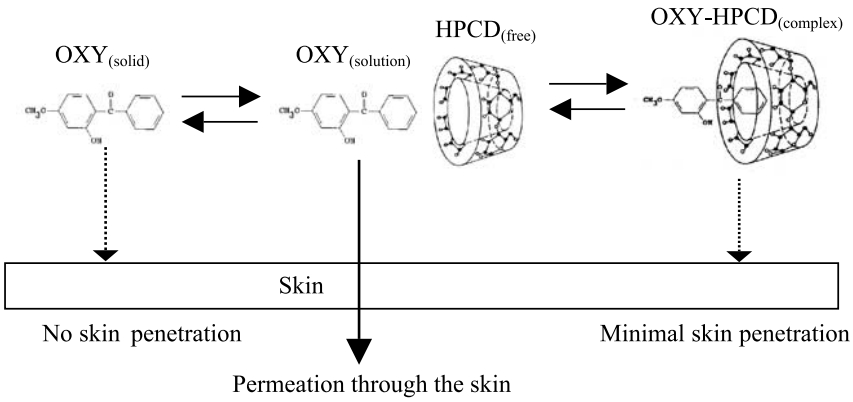

Figure 3. Schematic representation of the complexation and transdermal permeation of the oxybenzone-HPCD systems.

Edema

Inflammation is another acute biological response to UV radiation. The vasodilatation of cutaneous blood vessels results in erythema (reddening) and edema (swelling). As with SBC formation, peak erythema and edema occur approximately 24–48 hours postexposure.[28] Erythema is difficult to accurately quantify in the hairless mouse model due to multiobserver variability. Swelling, however, may be measured using a thickness gauge without subjective bias.

The extent of swelling 24 hours postUV exposure for the control and treatment groups is shown in Fig. 4. The degree of edema in the control group increased with increasing levels of UV radiation to a maximum of 29 μm at 2 MEDs. All treatment animals showed significantly less edema than the control group at both the suberythemal dose of 0.5 MED and at the higher exposures. Increasing the UV exposure from 0.5 to 1 MED resulted in an increased skin fold thickness for 0% and 10% HPCD formulations. Both the 5% and 20% HPCD formulations exhibited significantly less edema than the other HPCD-containing formulations investigated at 1 MED exposure.

Interestingly, at 2 MEDs of radiation, significantly less edema was found in skin treated with the 5% HPCD formulation compared to the 0% and 10% HPCD-containing experimental sunscreens. These results may be attributed to the accumulation of oxybenzone in the skin. In our previous in vitro study, the 5% HPCD formulation exhibited significantly greater oxybenzone accumulation in the skin two hours postapplication, although accumulation of the UV absorber at longer exposure times (4–24 hours) was greatest for the 10% HPCD formulation. The two-hour accumulation is relevant for this discussion as the animal exposure times did not exceed 2 hours. As previously mentioned, sunscreens must remain on the surface of the skin or the outermost epidermal layers in order to be effective. Since the inflammatory response initiates within the dermis, having oxybenzone present in the skin may help reduce swelling associated with UV exposure.

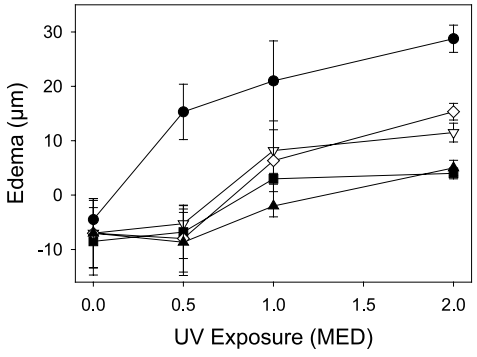

Figure 4. Influence of UV exposure on edema as a function of HPCD in the topical formulation. ●, Control; ▽, 0% HPCD; ■, 5% HPCD; ◇, 10% HPCD; ▲, 20% HPCD.

Lipid Melting Temperature

There is considerable evidence suggesting that UV radiation induces the formation of reactive oxygen species (ROS), resulting in damage to various components of the skin.[29] One significant consequence of ROS production in skin is lipid damage (oxidative

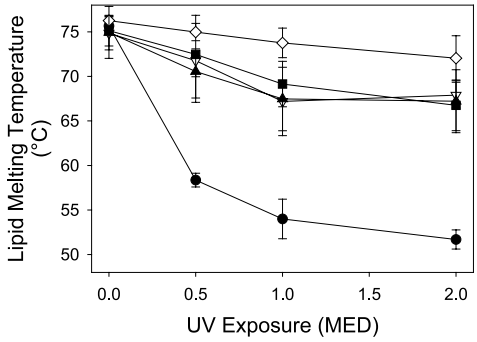

Figure 5. Influence of UV exposure on epidermal lipid melting temperature as a function of HPCD in the topical formulation. ●, Control; ▽, 0% HPCD; ■, 5% HPCD; ◇, 10% HPCD; ▲, 20% HPCD.

degradation of unsaturated free fatty acids and cholesterol),[30] which may alter the physicochemical structure of lipids allowing for increased fluidity.[31] In the current study, differential scanning calorimetry (DSC) was used to quantify the epidermal lipid melting temperature as a function of UV exposure. This thermoanalytical technique has previously been used to examine epidermal lipids following treatment with transdermal penetration enhancers. These topically applied chemicals temporarily reduce the barrier function of the skin by disrupting lipid packing, and DSC studies have correlated increases in both lipid fluidity and transdermal permeability with decreases in epidermal melting temperatures.[32,33]

In the current study, the authors hypothesized that UV exposure would decrease the lipid melting temperature of the mouse skin and that the application of sunscreens prior to UV radiation treatment would reduce this epidermal damage. Figure 5 shows the epidermal lipid melting temperature of both the control and treatment groups as a function of UV exposure. The lipid melting temperature of the epidermis of the control animals significantly decreased with UV exposure, even at suberythemal doses. All HPCD/oxybenzone-containing formulations reduced lipid damage, as evidenced by higher epidermal lipid melting temperatures, at all UV exposures investigated. No significant differences in epidermal lipid melting temperatures were found between any oxybenzone formulations tested at each exposure time investigated. While the 10% HPCD formulation exhibited the greatest protection against epidermal damage at the 2 MED exposure, these data were not significantly different from the other oxybenzone-containing formulations.

Comparison of HPCD-Oxybenzone Formulations and Commercial SPF 30 Sunscreen

The data generated in the current study were compared with an SPF 30 commercial sunscreen product. No statistical difference in the number of sunburn cells formed between skin treated with the 20% HPCD formulation and the SPF 30 sunscreen was found, as shown in Table 1. In contrast, the 5% HPCD formulation provided protection against edema similar to that of the commercial sunscreen and the 10% HPCD sunscreen exhibited protection against epidermal damage equivalent to the SPF 30 product. These data show that no single HPCD formulation provided photoprotection identical to that of the commercial sunscreen for all biomarkers of photodamage investigated in this study. It should be noted that the commercial sunscreen contained three UV-absorbing compounds in a w/o lotion while the HPCD/oxybenzone solutions and suspensions contained relatively small quantities of the single UV absorber. Thus, direct comparison of the data may not be appropriate. The authors performed this evaluation solely to provide

Table 1. Comparison of photoprotective effects of a commercial SPF 30 sunscreen and the HPCD-containing oxybenzone formulations at 2 MEDs.

Formulation	Sunburn cells (per cm)	Edema (μm)	Lipid melting temperature (°C)
Control	35.50 (5.32)	28.3 (4.0)	51.69 (1.08)
0% HPCD	15.75 (4.86)	11.7 (2.9)	67.89 (1.45)
5% HPCD	14.00 (3.92)	3.3 (2.6)	66.75 (2.85)
10% HPCD	14.00 (2.16)	12.7 (5.4)	72.04 (2.52)
20% HPCD	6.75 (2.63)	9.3 (7.5)	67.21 (3.53)
SPF 30 Sunscreen[a]	4.75 (2.75)	−3.7 (3.9)	74.57 (1.64)

Data reported as the mean (standard deviation).
[a]No-Ad® Ultra Sun Block lotion.

some point of reference for assessing the novel HPCD-containing sunscreens.

CONCLUSIONS

The findings from the current study demonstrated that cyclodextrin complexation does not prevent oxybenzone from interacting with light and that complexation may enhance the in vivo photoprotective effects of this model sunscreen. The 20% HPCD formulation exhibited better photoprotection of the skin, as evidenced by a reduction in sunburn cell formation. These data support the hypothesis that a drug reservoir was created on the surface of the skin. Ultraviolet-induced edema was minimized to the greatest extent by application of the 5% HPCD formulation. In comparison with a commercial SPF 30 sunscreen, no single HPCD-containing formulation exhibited equivalent photoprotection for all three biomarkers investigated.

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