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Research paper

Influence of hydroxypropyl-β-cyclodextrin on transdermal penetration and photostability of avobenzone

Jing Yang, Cody J. Wiley, Donald A. Godwin, Linda A. Felton *

University of New Mexico, Albuquerque, NM, USA

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Abstract

The objective of the present study was to determine the effects of hydroxypropyl-β-cyclodextrin (HPCD) complexation on the transdermal penetration and photostability of a model ultraviolet A (UVA) absorber, butyl methoxydibenzoylmethane (avobenzone), and to determine the influence of complexation on in vivo photoprotection. Avobenzone–HPCD complexation was demonstrated by differential scanning calorimetry. Formulations containing 0.12 mg/ml avobenzone and up to 30% (w/w) HPCD were prepared. Transdermal penetration was conducted using a modified Franz diffusion cell apparatus. As the concentration of HPCD was increased from 0% to 20%, transdermal permeation increased. Maximum flux occurred at 20% HPCD, where sufficient cyclodextrin was present to completely solubilize all avobenzone. When the concentration of HPCD was increased to 30%, transdermal penetration decreased, suggesting the formation of an avobenzone reservoir on the skin surface. Photostability of avobenzone was investigated under 100, 250, and 500 kJ/m^2 UVA irradiation. The 30% HPCD formulation was the most photostable, followed by 20%, 10%, and 0% formulations. In vivo, the 30% HPCD formulation afforded the best photoprotection, as evidenced by the lowest extent of sunburn cell formation and edema induction. This work indicates that inclusion of HPCD in sunscreen formulations may enhance photoprotection by reducing both skin penetration and photodecomposition of UV absorbers.

Keywords: Sunscreen; Avobenzone; Transdermal penetration; Photostability; Hydroxypropyl-β-cyclodextrin; Photoprotection

1. Introduction

Exposure of human skin to ultraviolet (UV) radiation leads to various skin pathologies, including acute inflammatory responses (i.e. erythema, edema) and chronic effects such as skin photoaging and skin cancer. Approximately 1,000,000 new cases of non-melanoma skin cancer (NMSC) and over 56,000 new cases of melanoma are diagnosed in the United States each year, resulting in nearly 10,000 deaths annually [1,2]. The use of sunscreens has been advocated as a good prevention against UV-induced skin damage. The most common active ingredients in sunscreen products are synthetic organic chemicals, which absorb UV light thereby reducing the dose of solar radiation that reaches the skin. Upon absorption of UV radiation, the activated UV absorbers dissipate the excited energy by heat, light, transferring to the surrounding molecules or undergoing photochemical reactions. Evidence from cell culture, animals, and human investigations has demonstrated that sunscreens can effectively protect against acute photodamage and chronic UV-induced skin cancer.

Butyl methoxydibenzoylmethane (avobenzone) is a commonly used UVA absorber (320–400 nm) with peak absorption at 360 nm (Fig. 1). Approved by the United States Food and Drug Administration (FDA) since 1998, avobenzone is one of only three active sunscreen ingredients available in the US that protect skin from the entire UVA spectrum (the other two are titanium dioxide and zinc oxide) [3]. Avobenzone can be easily photoinactivated

* Corresponding author. College of Pharmacy, University of New Mexico, 2502 Marble NE, Albuquerque, NM 87131, USA. Tel.: +1 505 272 2615; fax: +1 505 272 6749.
E-mail address: lfelton@unm.edu (L.A. Felton).

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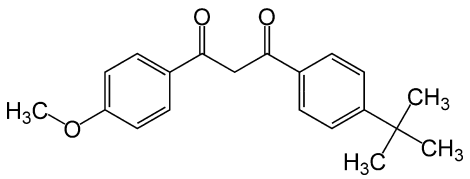

Fig. 1. Chemical structure of avobenzone.

and studies have shown a total decomposition of avobenzone when irradiated in a cyclohexane solution for 100 h using a 450 W mercury vapor lamp [4]. *p-tert*-Butylbenzoic acid and *p*-methoxybenzoic acid are the major degradation products, none of which has comparable UV absorption to the parent compound [5]. Moreover, Damiani et al. found a carbon-centered free radical was generated from the UV-induced decomposition of avobenzone and this free radical might be involved in DNA damage and lipid peroxidation [6].

Various methods have been developed to reduce photodecomposition of avobenzone: i.e. encapsulation in liposomes, loading in solid nanoparticles, or adding a photostabilizer into the formulation [7]. In the present study, the photostabilizing effect of cyclodextrin complexation was investigated. Cyclodextrins are crystalline, cyclic oligosaccharides consisting of 6, 7 or 8 (α-, β- or γ-cyclodextrin, respectively) glucopyranose units through α-1, 4-linkage with hydrophobic central cavities and hydrophilic exteriors. This unique shape allows for modifying the physicochemical properties of active substances by forming inclusion complexes through hydrogen bonding or van der Waals interactions. Cyclodextrins have been employed in pharmaceutical products to alter the solubility, stability, and bioavailability of various drugs [8]. Hydroxypropyl-β-cyclodextrin (HPCD) is a chemically modified cyclodextrin and is one of the most useful cyclodextrins in pharmaceutical applications due to its high aqueous solubility and inclusion capacity. This compound is too large and hydrophilic to readily penetrate into the skin [9]. HPCD has been subjected to extensive toxicological studies and is considered safe, with no tumor initiating or promoting effects [10]. The objective of the present study was to determine the influence of cyclodextrin complexation on the transdermal penetration and photostability of avobenzone. In addition, the corresponding in vivo photoprotective effects were investigated.

2. Materials and methods

2.1. Materials

Butyl methoxydibenzoylmethane (avobenzone) was supplied by Hoffmann-La Roche Ltd. (Geneva, Switzerland). 2-Hydroxypropyl-β-cyclodextrin (HPCD, 0.8 M substitution) and Brij®58 (polyoxyethylene (20) cetyl ether) were purchased from Sigma–Aldrich Inc. (Milwaukee, WI). Methanol was HPLC grade from Fisher Scientific Co. (Fair Lawn, NJ). Aqueous formaldehyde solution (37%) was purchased from EMD Chemicals Inc. (Gibbstown, NJ).

2.2. Avobenzone–HPCD interaction studies

A differential scanning calorimeter (DSC, Model 2920, TA Instruments, New Castle, DE) was used to demonstrate HPCD–avobenzone complexation. Avobenzone alone, a physical blend of the sunscreen and HPCD, and a dried powder obtained from evaporation of a solution of the two compounds were analyzed for the presence or absence of a melting transition. Samples of approximately 10 mg were sealed in aluminum pans and scanned at a rate of 10 °C/min from 0 °C to 120 °C. The modulation signal was set at 1.592 °C/min. The thermographs were analyzed using TA Instruments Universal Analysis software.

2.3. Aqueous solubility of avobenzone

The HPCD concentrations investigated in this study included 5%, 10%, 15%, 20%, and 30% (w/w). An excess amount of avobenzone was added to deionized water–HPCD solutions. The suspensions were covered with foil to prevent interaction with light and magnetically stirred for 48 h. The mixtures were then centrifuged at 3300 rpm for 10 min using a Fisher benchtop centrifuge (Pittsburg, PA) to separate the undissolved drug. An aliquot of the supernatant was analyzed for avobenzone content using high performance liquid chromatography (HPLC).

2.4. High performance liquid chromatography (HPLC) detection of avobenzone

The liquid chromatograph consisted of a binary pump solvent delivery system (Model P1500, Thermoseparations Products, Riviera Beach, FL), a 100-μl injection loop autosampler (Model AS 1000, Thermoseparations Products), and a variable-wavelength UV light absorbance detector (Model UV 1000, Thermoseparations Products). The analytical column was a 5-μm pore size, 4.6 × 150 mm C_{18} column (Alltech Associate, Inc., Deerfield, IL) 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 358 nm. The mobile phase was methanol:water (92:8) and the flow rate was 1 ml/min. The retention time was approximately 4 min.

2.5. Preparation of avobenzone-containing sunscreen formulations

Aqueous solutions and suspensions of avobenzone containing up to 30% (w/w) HPCD were prepared. The concentration of avobenzone was held constant at 0.12 mg/ml for all the formulations. This concentration was selected

based on the solubility of the UV absorber in a 20% HPCD solution. Thus, formulations containing less than 20% HPCD were suspensions while solutions were formed at higher HPCD concentrations (20% or 30% HPCD). The HPCD was dissolved in deionized water and then avobenzone was added. The formulations were covered with aluminum foil and stirred overnight using magnetic stir bars. These preparations were used in the in vitro transdermal penetration and photostability studies and the subsequent in vivo animal investigations.

2.6. Transdermal penetration of avobenzone

Hairless mouse skin was used for the in vitro transdermal penetration studies. Six-week-old female 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 dorsal skin (approximately 36 μm) was excised. Any extraneous subcutaneous fat was removed from the dermal surface and the skin was mounted onto modified Franz diffusion cells (donor surface area 0.64 cm^2; receptor volume 5.1 ml) containing isotonic phosphate buffer solution (pH 7.2) with 0.1% (v/v) 37% aqueous formaldehyde as a preservative and 0.5% (w/v) Brij®58 as a solubilizer [11]. The receptor fluid was maintained at 37 ± 0.5 °C and continuously stirred at 600 rpm using a magnetic stirrer. Following a 1-h hydration period, 200 μl of the avobenzone–HPCD formulation was applied to the skins ($n = 4$). Receptor fluid (300 μl) was withdrawn at specific time points over a 24-h period and immediately replaced with fresh buffer. These receptor fluid samples were then analyzed by HPLC for avobenzone content.

2.7. Avobenzone photostability

Since irradiation at selected wavelengths revealed that UVA was the most harmful wavelength responsible for photodecomposition of avobenzone [12], a UVA light source was used to irradiate the sunscreen samples. The UVA light (320–400 nm) was produced from an Oriel 1000 Watt Solar Ultraviolet Simulator (Oriel Corp., Stafford, CT) with a window glass blocking filter (WG320) to remove UVB radiation. Irradiation was 170 w/m^2 with the filter employed. The UVA doses used in this study were 100, 250, and 500 kJ/m^2, and the corresponding exposure times were 16, 40, and 80 min, respectively. These UVA doses were selected based on results of previously published etiology studies [13]. Exposure was measured using a Model 3D V2.0 Erythema UVA and UVB Intensity Meter (Solar Light Company, Philadelphia, PA). One hundred and twenty-seven microliters of the tested formulations was spread onto a 63.6 cm^2 (approximately 2 mg/cm^2) glass plate. Fifteen minutes after application, the plate was exposed to UVA radiation. Each sample was prepared in triplicate. For each UVA dose, another plate was kept in the dark as a control. After exposure, the samples were ultrasonically dissolved in 1500 μl of methanol for 1 min. The resultant solution was then transferred to a 13×100 mm glass tube and dried under nitrogen at room temperature. The dried extract was reconstituted with 1.27 ml of methanol and stored at −10 °C until analyzed by HPLC. All samples were protected from light both before and after irradiation.

The UV absorption spectra of the UVA-irradiated samples were measured using a UV–Visible Recording Spectrophotometer (UV 2000, Shimadzu Corp., Kyoto, Japan) scanned from 300 to 400 nm. Distilled water was used as a reference.

2.8. In vivo photoprotection of avobenzone–HPCD formulations

SKH-1 hairless mice were used for the in vivo photoprotection studies. The animal protocol was approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee. This animal model has been extensively used to investigate the histological and biochemical effects of UV radiation on skin and chronic UV-induced skin cancer [14]. Moreover, the skin of this animal model was used in the in vitro study to investigate transdermal penetration of avobenzone. Prior to experimentation, the 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–avobenzone formulations were applied at 2 mg/cm^2. Control mice were left untreated. After a 15-min drying period (as per FDA guidelines on sunscreen testing), the unrestrained mice were placed in a clear plastic box, covered with a barred lid and then exposed to 250 kJ/m^2 of UVA radiation.

Immediately prior to UVA exposure, the thickness of the skin folds at the back of the neck of four 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 thickness between the baseline and post-UV exposure data.

Sunburn cell (SBC) induction was measured in the animals 24 h after UVA exposure. The animal skins ($n = 4$) were removed, fixed in 37% formaldehyde solution, and sliced. Two non-sequential sections of the skin were mounted to a slide, hematoxylin and eosin stained, and subjected to microscopic examination (Olympus Reflected Light Fluorescence Microscope BH2-RFC, Olympus Optical CO., LTD, Tokyo, Japan) at 400× magnification. The number of SBCs per linear centimeter was calculated. Counts were done on 1.5-cm sections of interfollicular epidermis.

2.9. Data analysis

Statistical analysis employed a one-way analysis of variance (ANOVA) to determine if significant differences existed in the data. A $p < 0.05$ indicated significance. The analyses were performed using SPSS Sigma Stat Version 3.0 (SPSS Inc., Chicago, Illinois).

3. Results

3.1. Characterization of the avobenzone–HPCD complex

The ability of HPCD to form inclusion complexes with avobenzone was confirmed by differential scanning calorimetry (DSC). In general, when a guest molecule is incorporated into the cyclodextrin cavity, its melting point disappears [15]. The DSC thermographs of the investigational samples are shown in Fig. 2. Pure avobenzone powder exhibited an endothermic peak at 86.8 °C, corresponding to its melting point. The physical blend of avobenzone and HPCD showed a similar endothermic peak (87.7 °C), indicating an absence of interaction between avobenzone and HPCD upon simple mixing of the two solids. Powder obtained from solvent evaporation of an aqueous solution of HPCD and avobenzone exhibited no endothermic melt transition, demonstrating the molecular encapsulation of avobenzone into the HPCD cavity.

Fig. 3 shows the influence of HPCD complexation on the aqueous solubility of avobenzone. Avobenzone was practically insoluble (0.17 μg/ml) in pure water at room temperature, and the addition of HPCD resulted in a significant increase in solubility. The solubility of avobenzone in a 10% HPCD solution was determined to be 21.7 μg/ml, and increased sharply at higher HPCD concentrations (117.8 μg/ml in a 20% HPCD solution and 375.0 μg/ml in a 30% HPCD solution). The phase-solubility diagram exhibited a positive curvature (Ap-type) which, according to the literature, suggests that two complexes were formed, i.e. avobenzone–HPCD, 1:1 mole ratio and avobenzone–HPCD, 1:2 mole ratio, and these two inclusion complexes were in equilibrium in solution [16]. The stability constants ($K_{1:1}$ and $K_{1:2}$) for the complexation were calculated using Eq. (1), where $[S_0]$ is the intrinsic solubility of avobenzone, and $[S_t]$ and $[L_t]$ are the concentrations of avobenzone and HPCD in solution, respectively. The stability constants of the inclusion complexes were found to be 1580 M^{-1} for $K_{1:1}$ and 16 M^{-1} for $K_{1:2}$.

$$\frac{([S_t]-[S_0])}{[L_t]} = K_{1:1}[S_0] + K_{1:1}K_{1:2}[S_0][L_t]. \quad (1)$$

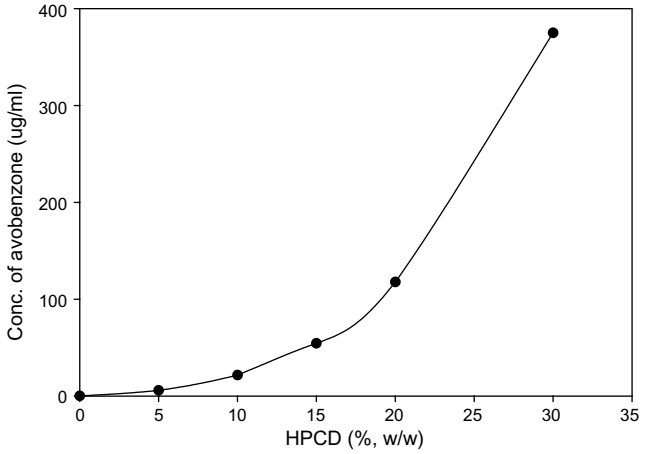

Fig. 3. Influence of HPCD concentrations on the aqueous solubility of avobenzone.

3.2. Influence of HPCD complexation on the in vitro transdermal penetration of avobenzone

SKH-1 hairless mouse skin was used to investigate the effects of HPCD complexation on the in vitro transdermal penetration of avobenzone. As shown in Fig. 4, the formulation containing 0% HPCD showed very low permeation through the skin (0.88 μg/cm^2 at 24 h). All HPCD-containing

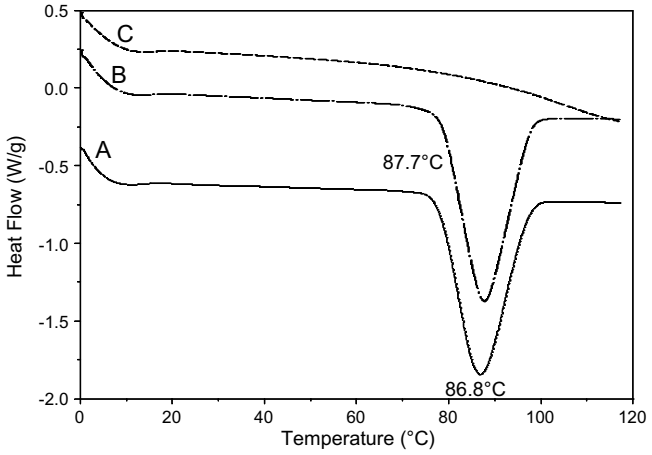

Fig. 2. Thermal analysis of A: avobenzone alone; B: a physical blend of avobenzone and HPCD in the dry state; C: avobenzone and HPCD prepared as a solution and precipitated.

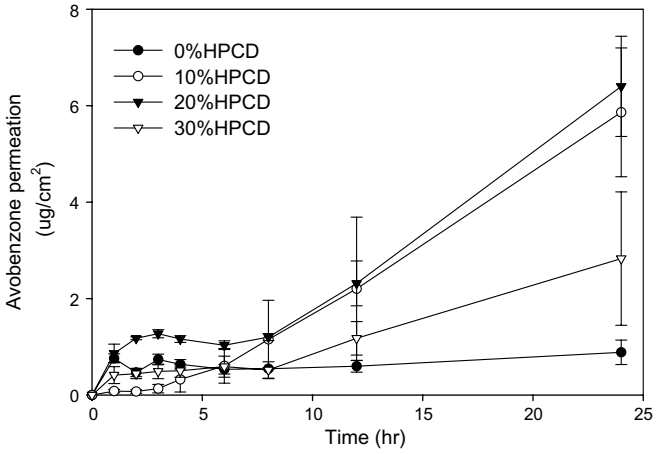

Fig. 4. Influence of HPCD concentrations on transdermal permeation of avobenzone.

formulations exhibited higher transdermal permeation compared to avobenzone alone (0% HPCD). The 20% HPCD exhibited the greatest permeation (6.40 μg/cm^2 at 24 h), and permeation was lower in both the 10% and 30% HPCD formulations (5.86 and 2.83 μg/cm^2 at 24 hour, respectively). Flux, representing the rate at which the drug penetrated through the skin, was calculated from the slope of the linear part from each permeation profile. The flux of avobenzone from the 0% HPCD formulation was low (0.0157 μg/cm^2/h), increased to the maximum from the 20% HPCD formulation (0.2431 μg/cm^2/h), and then decreased with addition of more HPCD (30% HPCD: 0.0756 μg/cm^2/h) (Fig. 5). These data are in agreement with the results reported by Felton et al., who showed a similar parabolic relationship between flux and HPCD concentrations [11].

Since the chemical must be dissolved in order to penetrate into the skin, solid avobenzone does not contribute to transdermal penetration, hence the low permeation of this UV absorber from the 0% HPCD formulation. The molecular size and hydrophilic character of the HPCD–avobenzone complex prevent any significant skin penetration from this species. Thus, only avobenzone in solution readily permeates through the skin. The 20% HPCD formulation produced maximum flux by maximizing the amount of free avobenzone available for permeation. The 30% HPCD concentration shifted the theoretical equilibrium of the complexation reaction towards the complexed form, thereby diminishing the amount of free avobenzone available for permeation and slowing the rate of skin permeation. In essence, the higher HPCD concentration created a sustained release delivery system on the skin surface. This is of great importance for the performance of sunscreen products since the UV-absorbing agents must remain in the outermost layer of the skin to be effective. In addition, since avobenzone generates free radicals when exposed to UV radiation, less direct contact between the UV absorber and the underlying living skin tissues may limit potential toxic or allergic reactions.

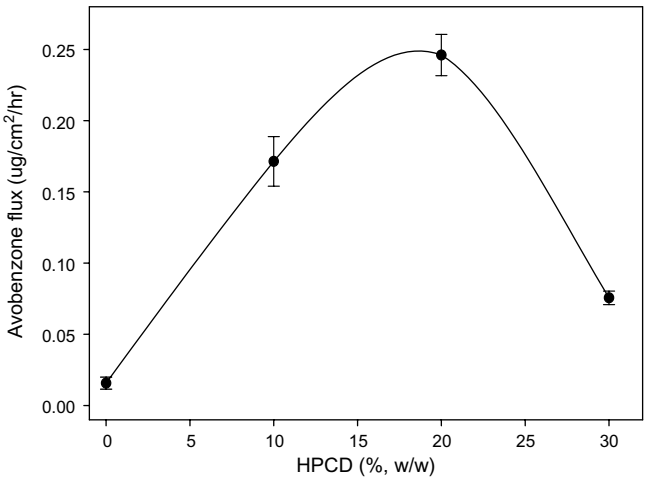

Fig. 5. Influence of HPCD concentrations on the flux of avobenzone.

In transdermal drug delivery systems, the active ingredient must partition from the vehicle prior to permeating into and through the skin. In this study, simple solutions and suspensions containing only avobenzone, HPCD, and water were used to investigate the effects of cyclodextrin complexation on transdermal penetration. The effects of various vehicles on skin permeation and interactions between a vehicle and HPCD were not evaluated in this study.

3.3. Photodegradation of avobenzone

First, the possible influence of complexation on the UV absorption ability of avobenzone was investigated by UV spectrophotometric examination of a 0.12 mg/ml avobenzone in 30% HPCD solution and the same concentration of avobenzone in methanol solution. As mentioned earlier, 30% HPCD was more than enough to complex all the avobenzone present in the formulation. As shown in Fig. 6, methanol did not absorb UV radiation within 300–400 nm. The 30% HPCD solution showed a slight absorption within this range. The spectrum obtained from the avobenzone–methanol solution exhibited a peak absorption at 358 nm with an absorbance of 2.32Abs (Fig. 6D). This characteristic absorption peak of avobenzone was also observed from the avobenzone-30% HPCD solution sample at 356 nm (2.43Abs). The slightly blue shift could be attributed to the formation of inclusion complexes in the sample and the more hydrophilic environment surrounding avobenzone [17]. Due to the contribution from HPCD, the absorbance of the avobenzone-30% HPCD solution was slightly higher than avobenzone from the methanol solution at equal concentration. These results suggest that HPCD complexation did not prevent avobenzone from reacting or absorbing UV radiation within 300–400 nm.

To investigate the influence of HPCD complexation on photodegradation of avobenzone, up to 30% (w/w) HPCD formulations were prepared and the concentration of

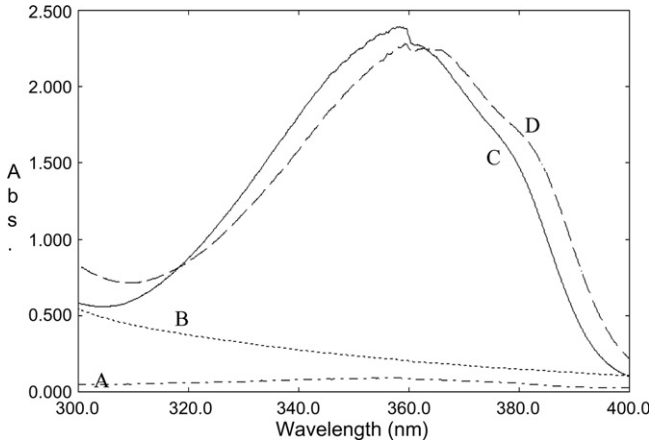

Fig. 6. UV absorption spectra of A: methanol; B: 30% HPCD; C: avobenzone in 30% HPCD (diluted to 12 μg/ml) and D: avobenzone in methanol (diluted to 12 μg/ml).

avobenzone was held constant at 0.12 mg/ml. A methanol solution of avobenzone at the same concentration served as a comparison. These formulations were exposed to 100, 250 or 500 kJ/m^2 of UVA, extracted, and then assayed by two methodologies: HPLC analysis and spectrophotometry. The extraction method was evaluated for linearity, precision, and accuracy. The calibration curve was found to be linear with a correlation coefficient of 0.994. The precision and accuracy of this analytical method were determined in avobenzone–methanol solutions. The precision value (coefficient of variance) was 0.26% and the accuracy value was 93.7%.

The recoveries of the investigational formulations were plotted against UVA exposures. The results obtained from the avobenzone in methanol and avobenzone in 0% HPCD samples were similar, and therefore only the result of the 0% HPCD sample is shown (Fig. 7a). Exposure of the 0% HPCD formulation led to a substantive reduction in the amount of avobenzone recovered, with only 23.1% of the absorber detected at 100 kJ/m^2 UVA exposure, 22.1% at 250 kJ/m^2, and 16.6% at 500 kJ/m^2. Increasing the dose of UVA did not result in a corresponding additional decomposition of this UV-absorbing agent, suggesting UV-induced decomposition of avobenzone did not follow a zero order reaction. The recoveries of avobenzone from the 10% HPCD formulation were similar to the 0% HPCD formulation, with 32.7% of the applied dose remaining at 100 kJ/m^2 UVA exposure, 30.7% at 250 kJ/m^2, and 24.6% at 500 kJ/m^2. As mentioned earlier, the concentration of HPCD at 10% was not sufficient to complex all avobenzone molecules in the formulation and therefore complexation offered limited protection against photo-induced degradation. HPCD at higher concentrations greatly suppressed photodecomposition. The recoveries of avobenzone increased almost twofold compared to avobenzone alone (0% HPCD formulation). The 30% HPCD formulation was the most photostable, as indicated by 74.6% recovery at 100 kJ/m^2 UVA exposure, 75.6% at 250 kJ/m^2, and 79.3% at 500 kJ/m^2. The high concentration of HPCD (30%, w/w), which was in excess of that necessary to complex all avobenzone (0.12 mg/ml), shifted the theoretical equilibrium of the complexation reaction towards the complexed form. Each avobenzone molecule had a greater chance to partition into the HPCD cavity than remaining as free in the solution. These data are in agreement with Scalia et al., who investigated the photostabilizing effect of avobenzone by HPCD complexation in a lotion (oil-in-water emulsion), and the recovery was found to be 82.7% after exposure with a 200 W Xenon solar simulator (>290 nm) for 4 h [18].

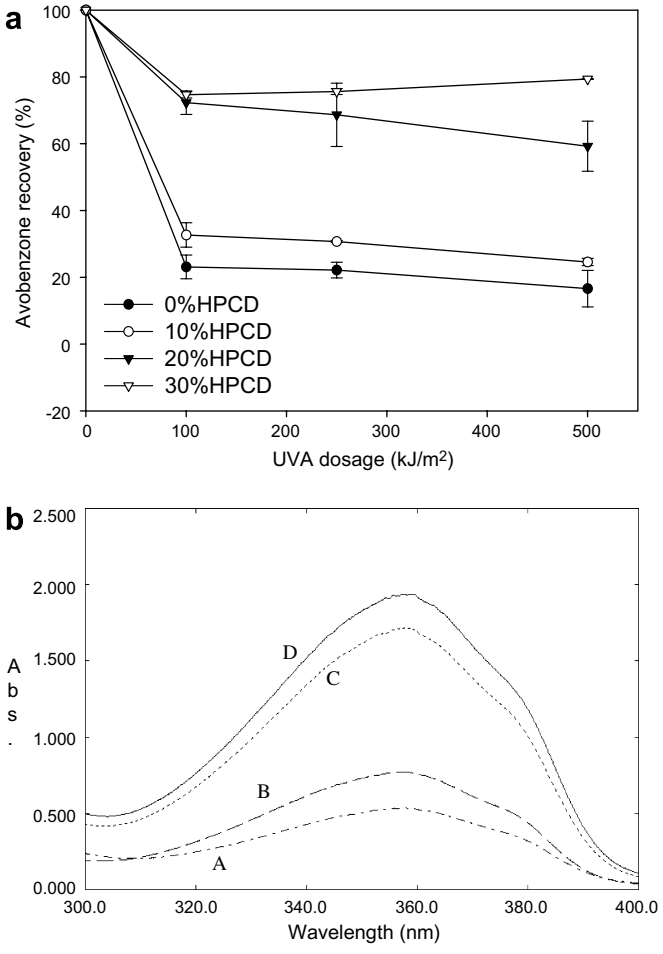

Fig. 7. (a) Recoveries of avobenzone as a function of UVA exposure, which were expressed as percentage of the initial dosage applied ($n = 3$). (b) UV absorption spectra of avobenzone (0.12 mg/ml) pre-irradiated with 250 kJ/m^2 UVA. Samples were 1:10 diluted before UV spectrophotometric examination. (A) 0% HPCD; (B) 10% HPCD; (C) 20% HPCD; (D) 30% HPCD.

UV spectrophotometry examination was performed as a complementary assay to evaluate UV absorption of avobenzone in the UVA pre-irradiated formulations. In Fig. 7b, the maximum absorption peaks were around 358 nm for all the pre-irradiated formulations, suggesting that the action spectra of avobenzone did not shift after UVA exposure. However, the degree of UV absorbance varied greatly. The 30% HPCD formulation showed the highest absorbance in this range (300–400 nm), followed by the 20% and 10% formulations, and the 0% HPCD formulation exhibited the lowest absorbance. These results are in agreement with the recovery data from the HPLC analysis, suggesting that the loss of UV absorbance was proportional to the photodecomposition of avobenzone.

3.4. Influence of HPCD complexation on the in vivo photoprotective effects of avobenzone

Different methods for testing UVA sunscreen protection have been investigated: i.e. DNA damage, p53 mutation, immunosuppression and free radical generation [19]. However, no agreed measures or formal protocols for UVA protection testing in the US have been defined [20]. In the current study, the photoprotective effects of avobenzone were evaluated in terms of acute UVA-induced skin inflammatory response (quantified by measuring the extent of skin edema induction) and epidermal cellular damage

(quantified by measuring the extent of SBC induction). UVA radiation was selected as the irradiation source based on the action spectrum of avobenzone (320–400 nm), thus avoiding the biological consequences induced by other wavelengths.

Inflammation is an acute biological response to UV radiation, characterized by skin erythema (reddening) and edema (swelling). Measurement of skin edema after UV exposure is a convenient and sensitive method for quantifying the extent of acute inflammation [21]. Skin edema is a frequently used biomarker to evaluate UVA-induced skin damage and peak formation has been found to be approximately 24–48 h following UV exposure [22]. Fig. 8 presents the extent of skin swelling 24 h after UVA exposure for the control and treatment animals. The increase in skin fold thickness as a measure of edema was 3.9 μm in the animals without sunscreen treatment. All avobenzone-containing formulations reduced the degree of skin edema (2.4 μm with 10% HPCD treatment, 1.9 μm with 20% HPCD, and nearly no edema detected with 30% HPCD). Significantly better protection was detected in the skin of animals treated with the 30% HPCD in comparison with any other treatment ($p < 0.05$).

SBCs are apoptotic keratinocytes that have absorbed a lethal dose of UV radiation and peak formation occurs 24 h following UV exposure [23]. Formation of SBCs has been studied extensively as an indication of acute photodamage, and this process is believed to protect against the accumulation of genetic damage and progression to skin cancer. When stained with hematoxylin and eosin, UV-exposed mouse skin showed cells with the classic appearance of SBC: an enlarged, pyknotic nucleus and dark vacuolated cytoplasm. As depicted in Fig. 9, skin sections of the animals without sunscreen treatment showed 8.3 ± 2.5 SBCs per cm of epidermis. Avobenzone treatment significantly inhibited this response ($p < 0.05$), with 6.7 ± 0.6 SBCs per cm in 0% HPCD group to 5.0 ± 1.0 SBCs per cm in the 30% HPCD group. Increasing the HPCD concentrations from 10% to 30% resulted in

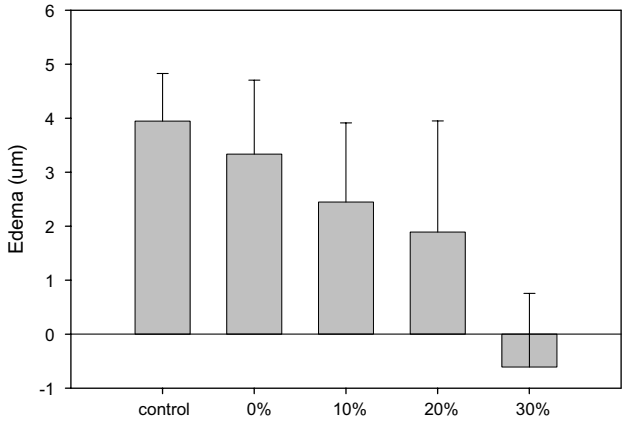

Fig. 8. Influence of UVA exposure (250 kJ/m^2) on edema as a function of HPCD in the formulations ($n = 4$).

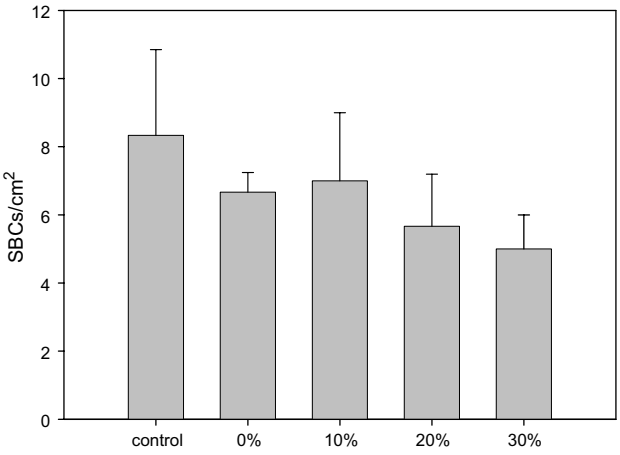

Fig. 9. Influence of UVA exposure (250 kJ/m^2) on the number of SBCs formed as a function of HPCD in the formulations ($n = 4$).

reduced SBCs counts, although no statistical difference in the number of epidermal SBCs was found between any of the avobenzone-containing formulations investigated. Compared with previous research, pure UVA radiation (250 kJ/m^2) was shown to be less capable of inducing SBCs than solar simulated UV (SSUV, UVA + UVB) light source, which induced approximately 28 SBCs per cm in the same animal model at the dose of 1 MED (1.4 kJ/m^2) [24]. Even the control animals in this study did not show a remarkable SBC induction, and therefore the protective effects afforded by the investigational sunscreens were not distinguishable. In this study, we also tested UVA-induced DNA damage and epidermal lipid damage as endpoints to evaluate the extent of photodamage. However, the 250 kJ/m^2 of UVA dose, which required 40 min of exposure, was not sufficient to induce a significant response in the control animals (data not shown). For measuring biological endpoints that require high intensity UVA exposure and long irradiation time, Garmyn and Roelandts suggest a photosensitizer be used in combination with UV exposure [25]. They made a comparable dose of UVA-induced skin damage detectable by pre-applying a photosensitizer, 8-methoxypsoralen (8-MOP), on the mouse skin to reduce the threshold dose for the induction of these biological endpoints. Using this technique, it may be possible to evaluate this sunscreen system in terms of more complete biological criteria, and this study would be necessary for further understanding of the efficacy and safety of these HPCD formulations.

4. Conclusions

The current study showed that HPCD complexed avobenzone, thereby significantly increasing the aqueous solubility of this UV absorber. The stability constant of the inclusion complexes was found to be 1580 M^{-1} for 1:1 avobenzone–HPCD complexation and 16 M^{-1} for 1:2 avobenzone–HPCD. Transdermal penetration studies using a

series preparations containing the same concentration of avobenzone (0.12 mg/ml) and up to 30% (w/w) HPCD showed that skin penetration was reduced when a high concentration of HPCD (30% w/w) was present in the formulation. When HPCD in excess of that necessary to complex avobenzone was added to the formulation, each avobenzone molecule had a greater chance of complexing with HPCD than penetrating into the skin. The UV absorber reservoir on the skin surface is expected to enhance the photoprotective effects of sunscreen products. In the photostability study, HPCD complexation was shown to significantly reduce photo-induced degradation of avobenzone. When HPCD (30%, w/w) was present in the formulation, the photodegradation of avobenzone was suppressed to the greatest extent. In vivo, the 30% HPCD formulation afforded better photoprotective efficiency, as evidenced by lower levels of sunburn cell and skin edema induction. This work indicates that HPCD complexation technology is a promising strategy to improve the photoprotective effects of sunscreen products.

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