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The risk of hydroquinone and sunscreen over-absorption via photodamaged skin is not greater in senescent skin as compared to young skin: Nude mouse as an animal model

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ABSTRACT

Intrinsic aging and photoaging modify skin structure and components, which subsequently change percutaneous absorption of topically applied permeants. The purpose of this study was to systematically evaluate drug/sunscreen permeation via young and senescent skin irradiated by ultraviolet (UV) light. Both young and senescent nude mice were subjected to UVA (10 J/cm^2) and/or UVB radiation (175 mJ/cm^2). Physiological parameters, immunohistology, and immunoblotting were employed to examine the aged skin. Hydroquinone and sunscreen permeation was determined by in vitro Franz cell. In vivo skin absorption was documented using a hydrophilic dye, rhodamine 123 (log $P = -0.4$), as a permeant. UVA exposure induced cyclooxygenase (COX)-2 and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) upregulation. Epidermal tight junction (TJ) were degraded by UVA. UVB increased transepidermal water loss (TEWL) from 13 to 24 g/m^2/h. Hyperplasia and inflammation, but not loss of TJ, were also observed in UVB-treated skin. UVA + UVB- and UVA-irradiated skin demonstrated similar changes in histology and biomarkers. UVA + UVB or UVA exposure increased hydroquinone flux five-fold. A negligible alteration of hydroquinone permeation was shown with UVB exposure. Hydroquinone exhibited a lower penetration through senescent skin than young skin. Both UVA and UVB produced enhancement of oxybenzone flux and skin uptake. However, the amount of increase was less than that of hydroquinone delivery. Photoaging did not augment skin absorption of sunscreens with higher lipophilicity, including avobenzone and ZnO. Exposure to UVA generally increased follicular entrance of these permeants, which showed two- to three-fold greater follicular uptake compared to the untreated group. Photoaging had less impact on drug/sunscreen absorption with more lipophilic permeants. Percutaneous absorption did not increase in skin subjected to both intrinsic and extrinsic aging.

1. Introduction

Skin aging can result from both chronological aging and extrinsic aging. Ultraviolet (UV) irradiation is the most significant cause of external aging (Naylor et al., 2011). Skin is mainly exposed to UVA (315–400 nm) and UVB (280–315 nm). Both types of irradiation elicit a range of cutaneous damage, accelerating the aging process. UVA is recognized as causing melanoma, wrinkling, and collagen breakdown (de la Coba et al., 2009). UVA can penetrate deeply into dermis (>300 nm). Exposure to UVB produces sunburn, epidermal hyperplasia, carcinogenesis, and transient inflammation (Sumiyoshi and Kimura, 2009), which is mainly absorbed by epidermis. Optimal care is necessary for aging skin to achieve skin disorder prevention and therapy. This may

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include topical application of sunscreens, antibiotics, anti-inflammatory drugs, and skin-lightening agents. We anticipated that UV exposure may affect the delivery of these agents through the skin because it alters skin structure. Most research testing drug absorption via the skin employs intact skin as a permeation barrier. The permeation profiles obtained from such studies are not appropriate for predicting their effect on diseased skin (Gattu and Maibach, 2010). It is essential, then, to determine whether such drugs can penetrate barrier-compromised skin or induce toxicity. We therefore aimed to establish permeation profiles of some drugs and sunscreens via aged skin.

Earlier investigations of skin aging have used senescent skin, skin exposed to UVA, or to UVB alone. In this project, we used both young and senescent skins, irradiated by UV for comparison. Besides respective UVA and UVB exposure, irradiation with different wavelengths may combine together since both UVA and UVB are the predominant wavelengths irradiated by sun exposure. We used hydroquinone, oxybenzone, avobenzone, and ZnO as model permeants. Topical hydroquinone is a common treatment for hyperpigmentation, which is a consequence of UV light exposure of skin (Hsieh et al., 2012). Sunscreens may protect against skin photoaging. Oxybenzone/avobenzone and ZnO are categorized as chemical and physical sunscreens, respectively. These permeants possess various physicochemical characteristics, which are helpful for comparison.

We used nude mice as an animal model for examining in vitro and in vivo percutaneous absorption of permeants. First, we examined physiological changes in skin produced by chronological aging and photoaging including transepidermal water loss (TEWL), pH, and skin lightness (L^*). The evidence of skin disruption was further determined by immunohistology and Western blotting. Although the stratum corneum (SC) is generally regarded as the predominant barrier against drug penetration, recent studies (Yamamoto et al., 2008; Liu et al., 2011) have shown that viable skin also plays an important part in resistance as a permeation barrier. Thus, we detected biomarkers from different skin layers, including cyclooxygenase (COX)-2, E-cadherin, cytokeratins, and heat shock proteins (HSPs). This is the first report investigating the effect of UVA and UVB exposure on skin absorption and follicular uptake of drugs and sunscreens.

2. Materials and methods

2.1. Materials

Hydroquinone (molecular weight, MW = 110, log P = 0.59), oxybenzone (MW = 228, log P = 3.79), avobenzone (MW = 310, log P = 4.56), ZnO powder, ZnO nanoparticles (<100 nm), and propylene glycol (PG) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ethyl cyanoacrylate superglue was obtained from 3 M (Taipei, Taiwan). 4′,6-Diamidino-2-phenylindole (DAPI) was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Polyvinylidene fluoride (PVDF) filter (0.45 μm) was supplied by Millipore (Billerica, MA, USA).

2.2. Animals

Female nude mice (ICR-Foxn/^{nu} strain) were purchased from Taiwan's National Laboratory Animal Center (Taipei). Mice aged 8 and 24 weeks old were categorized as young and senescent, respectively. A laboratory diet and water were given ad libitum before experiments. The mice were treated according to the Ethical Guidelines of the Animal Center, and the experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Chang Gung University.

2.3. UVA and UVB treatments

A Bio-Sun system illuminator (Vilber Lourmat, Marne-la-Vallée, France) was used to produce UVA and UVB radiation. Two UV sources were utilized to irradiate the peak wavelengths of 365 and 312 nm for UVA and UVB, respectively. The distance between the nude mice and the lamps was 10 cm. The spectral irradiance was 10 J/cm^2 and 175 mJ/cm^2 for UVA and UVB, respectively. The dorsal region of the mouse was irradiated with UVA every other day for three days. They were exposed to UVB once a day for five days. The duration of UVA and UVB exposure was 100 and 1 min each day, respectively. Irradiation with UVA and UVB was also combined for treating skin if necessary.

2.4. Macroscopic evaluation

All examinations were performed 1 h after the end of the UV irradiation course. These measurements included TEWL, skin pH value, and lightness (L^*). TEWL was calculated by a Tewameter® (TM300, Courage and Khazaka, Köln, Germany) to determine the water evaporation rate (g/m^2/h). Skin pH was detected by a Skin-pH-Meter® (PH905, Courage and Khazaka). For measurement of skin lightness, we used a spectrocolorimeter (CD100, Yokogawa, Tokyo, Japan) to precisely determine the color of the skin.

2.5. Histological analysis

Skin samples were excised from the nude mice after different treatments, then fixed in 4% buffered formaldehyde at pH 7.4. Samples were dehydrated with ethanol and embedded in paraffin wax. The skin was sectioned at 3 μm-thick and stained with hematoxylin and eosin (H&E). Immunohistochemistry was performed on the specimens as described in a previous study (Lin et al., 2009). For immunostaining, we used the polyclonal antibodies of COX-2 to observe possible skin inflammation and dermal extracellular matrix degradation. All biopsies were evaluated under IX81 microscopy (Olympus, Tokyo, Japan).

2.6. Immunofluorescence staining of E-cadherin

Skin subjected to UV was excised from the dorsal region of nude mice. The skin specimens were then fixed in buffered formaldehyde solution and cut vertically. We used immunofluorescence analysis of E-cadherin to examine tight junction (TJ) in the epidermis. The skin was incubated with a primary anti-E-cadherin antibody at room temperature for 1 h. Immunoreactivity was detected with an Alexa Fluor 594 goat anti-rabbit immunoglobulin antibody (Invitrogen, Carlsbad, CA, USA). DAPI at 100 ng/ml was also pipetted to specimens for staining cell nuclei.

2.7. Western blotting

Proteins were separated on 10% denatured gels and transferred to PVDF membrane. The membrane was incubated for 1 h with a blocking solution of 5% non-fat milk–TBST solution, followed by immersion overnight in the same solution with respective antibodies against pan-cytokeratin, HSP27, and HSP60 at 4 °C. After washing with TBST, peroxidase-labeled anti-rabbit immunoglobulin G was added and incubated for 2 h. Enhanced chemiluminescence (PerkinElmer, Waltham, MA, USA) was used for detecting proteins. Band intensity was quantified using GeneTools Image Software (Syngene, Cambridge, UK). We used the band of β-actin as an internal control.