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Research paper

Human skin penetration and local effects of topical nano zinc oxide after occlusion and barrier impairment

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ABSTRACT

Public health concerns continue to exist over the safety of zinc oxide nanoparticles that are commonly used in sunscreen formulations. In this work, we assessed the effects of two conditions which may be encountered in everyday sunscreen use, occlusion and a compromised skin barrier, on the penetration and local toxicity of two topically applied zinc oxide nanoparticle products. Caprylic/capric triglyceride (CCT) suspensions of commercially used zinc oxide nanoparticles, either uncoated or with a silane coating, were applied to intact and barrier impaired skin of volunteers, without and with occlusion for a period of six hours. The exposure time was chosen to simulate normal in-use conditions. Multiphoton tomography with fluorescence lifetime imaging was used to noninvasively assess zinc oxide penetration and cellular metabolic changes that could be indicative of toxicity. We found that zinc oxide nanoparticles did not penetrate into the viable epidermis of intact or barrier impaired skin of volunteers, without or with occlusion. We also observed no apparent toxicity in the viable epidermis below the application sites. These findings were validated by *ex vivo* human skin studies in which zinc penetration was assessed by multiphoton tomography with fluorescence lifetime imaging as well as Zinpyr-1 staining and toxicity was assessed by MTS assays in zinc oxide treated skin cryosections. In conclusion, applications of zinc oxide nanoparticles under occlusive in-use conditions to volunteers are not associated with any measurable zinc oxide penetration into, or local toxicity in the viable epidermis below the application site.

1. Introduction

There is a lack of consensus about the safety of zinc oxide nanoparticles (ZnO NP) applied topically as a transparent and effective broad spectrum ultraviolet filter, with a range of views expressed by regulatory authorities [1–3], consumer groups [4,5] and in the scientific literature [6–9]. These safety concerns should be balanced against the potential benefits provided by ZnO NP-containing sunscreens in preventing adverse outcomes resulting from excessive ultraviolet radiation exposure, which include sunburn and photo-allergy due to accelerated skin ageing, immunosuppression and an increased risk of developing skin cancer [10].

One perception is that ZnO NP and other small inorganic NP currently used in commercially available sunscreens may penetrate deep into the skin and cause a variety of adverse effects [11]. Topically applied NP occasionally reach the viable epidermis [12,13] and zinc ions have even been found in human blood and urine after topical ZnO NP application [14,15], as well as in the SC and upper epidermis of sunburned pig skin [16]. Further, after short-term *in vitro* exposure above 15 μg mL^{-1}, ZnO NP reduce keratinocyte viability [17]. An alternative view [18], supported by several *in vivo* human studies, is that ZnO NP topically applied to unoccluded intact human skin penetrate only into the superficial layers of the stratum corneum (SC), with no penetration to the viable epidermis (VE), both *in vitro* and *in vivo* [1–3].

There is a clear need to determine the *in vivo* penetration and local skin safety of ZnO or other NP in humans after topical

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application under "in-use" conditions. Of particular importance are the "in-use" conditions of abrasion and increased skin hydration that are commonly encountered during work, sport and beach activities. The current safety evaluation of ZnO NP applied to skin has focussed on a systemic margin of safety, noting that the body has homeostatic mechanisms to deal with excessive zinc ion (Zn^{2+}) exposure [19]. We are not aware of any *in vivo* human study evaluating the local safety of ZnO NP applied to the skin under occlusive conditions without or with impaired skin barrier function.

Here we explore the impact of occlusion on the topical penetration and local toxicity of ZnO NP into the viable epidermis of intact and barrier-impaired human skin *in vivo*.

2. Materials and methods

2.1. ZnO nanoparticles

Uncoated and coated ZnO NP (Z-COTE® and Z-COTE® HP1 respectively; BASF, Ludwigshafen, Germany) were re-suspended in caprylic/capric triglyceride (CCT) for application to the skin. Transmission electron microscopy (TEM) was performed on a JEOL 1400 microscope (JEOL Ltd, Tokyo, Japan) operating at 120 kV. Images were acquired using a TVIPS F416 CCD camera (TVIPS, Gauting, Germany) and analysed by ImageJ to determine particle sizes.

2.2. Application of ZnO NP to the skin of human volunteers

The study was carried out on 10 healthy subjects between the ages of 20–40 years with undamaged skin and no history of cutaneous disease. Skin was considered undamaged if TEWL was less than 20 g m^{-2} h^{-1} [20,21]. The volunteers gave informed consent and experiments were conducted with approval from the University of Queensland Human Research Ethics Committee (No. 2008001342), in accordance with Declaration of Helsinki protocols. Four treatment areas marked on each subjects' volar forearm were either (i) untreated, (ii) treated with vehicle (CCT), (iii) barrier-impaired by tape stripping before treatment with 10% w/v ZnO NP in CCT, or (iv) treated with 10% w/v ZnO NP in CCT with the barrier intact, with the standard SPF-testing dose (2 mg cm^{-2}) [22]. Areas on one arm were occluded by placing two adhesive dressings (Johnson & Johnson Pacific Pty Ltd., Sydney, Australia) over the sites of application. Sites remained unoccluded on the other arm. Coated ZnO NP were used in five subjects and uncoated ZnO NP in the other five. The barrier-impaired areas (4 cm^2) were tape-stripped 20 times with D-Squame tape (CuDerm, Dallas, US) immediately prior to administration of the ZnO NP suspensions. All treatments were applied for 6 h, after which the excess ZnO NP were wiped off using a surgical swab moistened with water.

2.3. Multiphoton-FLIM imaging of autofluorescence and ZnO NP in the skin of human volunteers

A DermaInspect® system (JenLab GmbH, Jena, Germany) was used for multiphoton tomography (MPT) as previously described [21]. The skin was imaged at three depths from the skin surface, corresponding to the SC (~5–10 μm), stratum granulosum (SG; ~15–20 μm) and stratum spinosum (SS; ~25–30 μm). Fluorescence lifetime decay data from the MPT-FLIM images were analysed with SPCImage 5.0 software [23] after correction for the instrument response function.

ZnO NP were quantified by their quasi-continuous accumulated photoluminescence on laser pulsing as illustrated in the sketch Fig. 1A and B. Standard curves were created for uncoated and coated ZnO NP in CCT (50, 100, 500 μg mL^{-1}, and 1, 5 and 10 mg mL^{-1}) on glass slides using bh SPCImage. GraphPad Prism Ver 6.05 (GraphPad Software, Inc., La Jolla, CA) was used to conduct non-linear regression of standard curves.

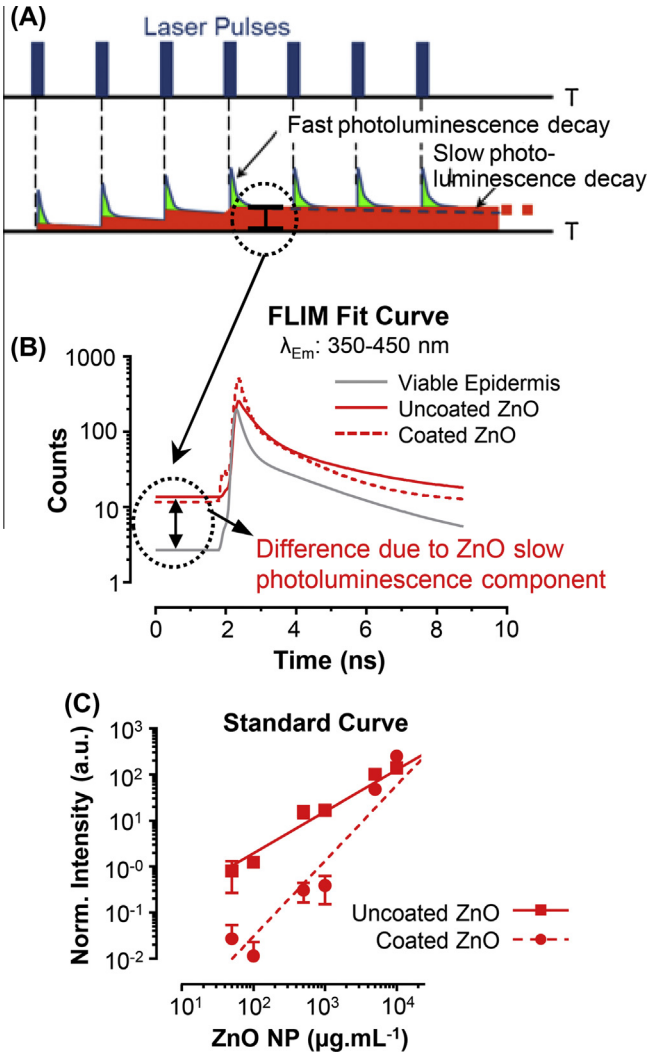

Fig. 1. (A) ZnO NP fast and slow decaying photoluminescence, the latter accumulating on repeated laser pulsing to create enhanced background signal related to ZnO NP concentration, defined as '*offset*$_{5-25}$'. (B) Fluorescence decay fit curves of representative pixels from the viable epidermis (grey), uncoated (red solid line) and coated (red dashed line) ZnO NP within *in vivo* human skin after topical application. (C) Standard curves for uncoated (solid line) and coated ZnO NP (dashed line), showing normalised signal intensities plotted against ZnO NP concentrations in μg mL^{-1} on a log–log scale. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.4. Data analysis

FLIM data were derived from the fluorescence lifetime decay curves registered in 256 time channels for each pixel using bh SPCImage 5.0 software [23], as previously described [21].

For autofluorescence metabolic analysis, a distinct region of interest was defined to encapsulate the viable cells within the SG and SS using SPCImage. To visualize, isolate, and measure the level of ZnO NP distributed in the skin and within targeted regions of interest, the baseline value of the decay curve of each pixel was used. These values ('*offset*' value in bh SPCImage) were pseudo-coloured in red to create images.