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A quantitative radioluminographic imaging method for evaluating lateral diffusion rates in skin

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ABSTRACT

A method is presented for measuring the lateral diffusion coefficients of exogenously applied compounds on excised skin. The method involves sequential high resolution imaging of the spatial distribution of β-radiation associated with [^{14}C]-labeled compounds to monitor the development of the concentration profile on the skin surface. It is exemplified by measurements made on three radiolabeled test compounds – caffeine, testosterone, and zinc pyrithione (ZnPT) – administered as solutions. Lateral diffusivity is expected to be an important determinant of the topical bioavailability of ZnPT, which is characteristically administered as a fine suspension and must reach microorganisms in molecular form to exert biocidal activity. Application of the test compounds at levels below and above their estimated saturation doses in the upper stratum corneum allows one to distinguish between diffusion-limited and dissolution rate-limited kinetics. The effective lateral diffusivities of the two chemically stable reference compounds, caffeine and testosterone, were $(1–4) \times 10^{-9}$ cm^2/s and $(3–9) \times 10^{-9}$ cm^2/s, respectively. Lateral transport of [^{14}C] associated with ZnPT was formulation-dependent, with effective diffusivities of $(1–2) \times 10^{-9}$ cm^2/s in water and $(3–9) \times 10^{-9}$ cm^2/s in a 1% body wash solution. These differences are thought to be related to molecular speciation and/or the presence of a residual surfactant phase on the skin surface. All values were greater than those estimated for the transverse diffusivities of these compounds in stratum corneum by factors ranging from 250 to over 2000. Facile lateral transport on skin, combined with a low transdermal permeation rate, may thus be seen to be a key factor in the safe and effective use of ZnPT as a topical antimicrobial agent.

1. Introduction

The permeability of the skin's outer layer, the stratum corneum (SC), to exogenously applied chemicals has been widely researched. The vast majority of the studies have investigated the transverse transport of organic solutes of importance to topical and transdermal drug delivery [1] and dermal risk assessment [2]. Less is known regarding lateral transport on skin or the transport of organometallic compounds, yet both are important. We consider the example of zinc pyrithione (ZnPT), a broad-spectrum biocide that is FDA-approved to treat dandruff and seborrheic dermatitis, but is also of interest as a routine topical antimicrobial agent [3,4].

ZnPT is commonly dispersed in complex aqueous-based formulations and is deposited on the skin surface as a particle due to its sparse solubility in water (5–15 ppm) [5]. The efficacy of anti-dandruff activity (in large part directed at the yeast, *Malassezzia globosa*) is positively correlated with the amount of ZnPT platelets deposited and retained on the scalp after rinsing [6] and inversely related to the particle size over a certain application range [7]. ZnPT has long been known to persist on the skin surface following deposition from a variety of surfactant vehicles [8] and shampoo matrices [9]. Recently it has been shown that long-term bioavailability is optimized by coacervate technology [10] which enhances substantivity of ZnPT in the superficial layers of the SC and follicular infundibulum where recolonization of the microorganisms occurs [11,12]. It is further thought that dissolution, chemical speciation and transport on the skin surface play a role in this activity [13], yet there is little quantitative information regarding these processes. The present study is directed at elucidating lateral transport.

ZnPT in shampoo formulations has a long history of safe use by humans, supported by independent regulatory evaluations [14]. In this regard, numerous reports exist in the literature evaluating the transverse permeation of ZnPT, or components thereof, and other pyrithione conjugates through the skin, cf. [5] and references therein. Many of these studies have been performed to determine systemic exposures of pyrithiones in animal models for use in the human dermal risk assessment of ZnPT. Collectively, the animal studies suggest that ZnPT dissociates and may react further during transport through the skin. In vivo studies in rabbits and rats using ZnPT labeled with ^{65}Zn, ^{14}C or ^{35}S demonstrate that small/negligible amounts of zinc permeate the SC relative to the carbon or sulfur moieties [15,16]; thus, permeation may

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be largely associated with the organic portion of the molecule. For lateral transport, no quantitative data are available for ZnPT.

To begin to address this question we have studied the lateral transport of carbon associated with [^{14}C]-ZnPT on isolated samples of human SC using a quantitative radioluminographic imaging method also known as phosphorimaging. This non-destructive assay can monitor and spatially resolve the analyte distribution profile in the plane of the skin surface at various incubation times. To gain experience with – and confidence in – this method we included two chemically stable, well studied reference compounds, caffeine and testosterone [17], in the investigation. The phosphorimaging method is complementary to two other recently developed techniques in which either fluorescence after photo-bleaching (FRAP) [18] or high resolution infrared (IR) spectroscopy [19] was used to obtain similar information on lateral transport rates in skin. Development of these molecular imaging procedures overcomes many of limitations imposed by previous methods that rely on the analysis of dissected tissues [20–25].

A scientific side benefit of the study is the possibility it presents to better characterize microtransport processes within the SC. The tissue is highly anisotropic, with layers of broad, flattened corneocytes separated by thin layers of lamellar lipids [26]. The upper layers of the SC lose their lipid content and desmosomal bridges between the corneocytes in the process of desquamation [26,27]. It is evident from this structure that the tissue should exhibit different rates of diffusion in transverse and lateral directions, and that the degree of anisotropy will depend on the properties of the protein and lipid phases. Quantitative lateral diffusion measurements provide a hitherto unavailable test of these details. A comparison of the results with predictions from the microscopic transport model of Wang et al. [28,29] is presented.

2. Materials and methods

2.1. Materials

Split thickness human cadaver skin, dermatomed to a nominal thickness of 300 μm, from the posterior torso or leg was obtained from the New York Presbyterian Hospital Skin Bank (New York, NY). This work with de-identified human tissues was considered by the University of Cincinnati Institutional Review Board to be exempt from human subjects regulations. [1-Methyl-^{14}C]-caffeine (99% pure 55 mCi/mmol in ethanol), [4-^{14}C]-testosterone (99% pure 53 mCi/mmol in ethanol), and carbon-14 autoradiography standards (0.00971–229 nCi/mm^2) were purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Solid [^{14}C]-ZnPT (96% pure 149 mCi/mmol) was synthesized by Lonza Group Ltd. (formerly Arch Chemicals) and donated by The Procter & Gamble Company (Cincinnati, OH). Type II-S trypsin from porcine pancreas, type II-S trypsin inhibitor from soybean, Dulbecco's phosphate buffered saline (PBS), sodium carboxymethyl cellulose (average MW ~ 250,000), lithium sulfate (≥98%), sodium acetate trihydrate (≥99.5%), acetic acid (≥99.7%), and Kodak® Intensifying Screen Cleaner were purchased from Sigma-Aldrich® Co. LLC (St. Louis, MO). Soluene-350® and Ultima Gold® XR scintillation fluid were purchased from Perkin-Elmer Life Sciences (Boston, MA). Deionized (DI) water was prepared using a Milli-Q® purification system (Millipore Corporation, Billerica, MA). Ivory® Original Scented Body Wash and Scotch® Magic™ Tape were purchased commercially. The Storage Phosphor Screen (BAS-IP MS 2025 E) was purchased from GE Healthcare Life Sciences (Buckinghamshire, UK).

2.2. Preparation of stratum corneum samples

Split thickness skin specimens were stored at −80 °C in Roswell Park Memorial Institute (RPMI)-1640 solution preserved with oxacillin sodium and gentamicin. Prior to use, the skin was rapidly thawed and thoroughly rinsed in room temperature DI water. The SC was isolated by heat separation and enzymatic digestion. The heat separation process was performed by soaking the skin sections in 57 °C DI water for 90 s followed by physical removal of the epidermis by carefully peeling it from the dermis with forceps. Following dissection of the epidermis, the viable epidermis was digested with a PBS solution containing 0.01% trypsin which was incubated overnight in a closed petri dish at 4 °C. Removal of the digested tissue from the underside of the epidermis was achieved by washing with PBS and gentle brushing with cotton swabs. Isolated SC specimens were immediately treated with a PBS solution containing 0.01% trypsin inhibitor and rinsed three times with DI water. They were then cut into approximately 1.5 cm^2 pieces and dried flat on plastic microscope slide coverslips in a desiccator containing Drierite®. SC samples were stored in a desiccator at −20 °C for a maximum of six months to maintain skin integrity.

2.3. Dose solution preparation

Solutions containing [^{14}C]-caffeine and [^{14}C]-testosterone were prepared to yield concentrations below, equal to, and over the amount of permeant required to saturate the SC (M_{sat}, μg/cm^2) for a 2 μL volume applied to partially hydrated skin. Calculations of these concentrations were based on the surface area of the evaporated dose (cm^2) and the following set of equations:

$$M_{sat} = C_{sat} \times h_{dep} \tag{1}$$

$$C_{sat} = S_w \times K_{sc/w} \tag{2}$$

$$K_{sc/w} = 0.04 \times K_{o/w}^{0.81} + 0.0359 + 4.057 \times K_{o/w}^{0.27}. \tag{3}$$

In Eqs. (1)–(3), C_{sat} is the permeant solubility in the SC (g/cm^3), h_{dep} is the initial deposition depth of the permeant (assumed to be 10% of total SC thickness [27] ~1.34 μm for partially hydrated SC), S_w is the water solubility of the permeant at skin temperature (32 °C), and $K_{sc/w}$ is its SC/water partition coefficient [27,30]. The concept of a deposition depth is associated with the desquamating layers of the SC and has been useful for describing the skin disposition of small doses of other topically-applied compounds [27,31]. [^{14}C]-Caffeine was stored in ethanol or diluted by 50% with DI water to increase the surface tension of the droplet on the skin and yield a smaller dose area. Due to limited aqueous solubility, [^{14}C]-testosterone was stored in ethanol.

Based on the sparse solubility of ZnPT in vehicles suitable for topical application, a variety of dosage forms for [^{14}C]-ZnPT were employed in order to elucidate the influence of concentration and dissolution on the lateral diffusion rates. These solutions included unsaturated and saturated solutions in water containing 1% (w/v) carboxymethyl cellulose or 1% (w/w) buffered (pH = 5.3, 100 mM acetate buffer) body wash solution. Unsaturated solutions were prepared to mimic doses equivalent to the clinically relevant rinse-off deposition amounts for ZnPT on the arms, 0.1 μg/cm^2, as well as the calculated saturation dose, M_{sat} = 0.011 μg/cm^2, while saturated solutions provided dose concentrations around the in vivo deposition amounts of ZnPT found on the scalp for a shampoo and shampoo tonic matrix, 0.5 μg/cm^2 and 0.8 μg/cm^2 respectively (E.D. Smith, personal communication).

2.4. Topical administration of radiolabeled solutions

Partially hydrated SC in vivo has an average water content of about 30% (w/w) [32]. This level of SC hydration can be attained ex vivo when the tissue is equilibrated in a closed vessel with a relative humidity of 85% [32,33]. To achieve this, the isolated SC samples were equilibrated overnight at 32 °C in a vessel containing an aqueous solution saturated with lithium sulfate [34]. The temperature was maintained by placing the vessel into an Air-Shields Isolette C100 QT-EC infant warmer incubator (Eaton® Corporation, PLC; formerly Vickers®). Due to photostability issues with ZnPT, the incubator was covered in black construction paper to prevent degradation.