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Determination of permeation pathways of hydrophilic or hydrophobic dyes through the mammary papilla

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ABSTRACT

The transport pathways and permeation kinetics of lipophilic and hydrophilic fluorescent dyes through porcine mammary papillae were visualized and quantified. Porcine mammary papillae, removed from full-thickness abdominal tissue, were positioned in a Franz diffusion cell for passive diffusion studies. Solutions containing the fluorescent dyes were applied topically for time periods ranging from 30 min to 48 h. Dye concentrations in tissue and Franz diffusion compartments were analyzed using fluorescence microscopy and fluorimetry. Fluorescence micrographs elucidated two permeation pathways, transepidermal and transductal. Hydrophilic sulforhodamine B predominantly penetrated via the transepidermal route, while lipophilic nile red diffused mainly by the transductal route. An almost 4-fold higher amount of sulforhodamine B was retained within the nipple over time compared to nile red, despite both dyes permeating through the tissue at similar rates. Diffusion through the porcine nipple was 500-fold higher than through adjacent skin for both dyes, likely attributable to the two mammary ducts which provide an entry point and transport route through the tissue. These results, generated from both qualitative and quantitative evidence at a micro and macro scale, demonstrate that the mammary ducts provide a direct pathway that contributes significantly to passive transport through the nipple, particularly for lipophilic molecules.

1. Introduction

Invasive breast cancer is the most frequently diagnosed cancer, with an estimated 250,000 women diagnosed annually (American Cancer Society, 2017). An additional 167,000 women will be diagnosed with high-risk, pre-malignant or non-invasive breast lesions, including atypical hyperplasia, lobular carcinoma in situ, and ductal carcinoma in situ (American Cancer Society, 2017; Hartmann et al., 2015). In the earliest stages, pre-malignant or malignant cells are localized within the ducts of the breast. Yet, the treatment approaches for these women are the same as those with invasive cancer and impose psychological detriments resulting from a life-altering, irreversible mastectomy or unnecessary systemic side effects as a result of oral treatments on women who may never develop invasive breast cancer. Moreover, the substantial side effects are a contributing factor in the low adoption and adherence rates of chemopreventive drugs. Only 16% of women identified as high-risk for developing breast cancer adopt primary preventive therapy and of those, < 65% complete the full 5-year regimen (Smith et al., 2016). Therefore, despite the availability of effective drugs, their systemic delivery route has limited their use in the prophylactic setting.

A local drug delivery strategy, however, would maximize drug exposure within the breast, while minimizing systemic exposure, thereby resolving the primary issue of side effects while still reaping the benefits of a preventive treatment. A variety of approaches have been suggested to localize drug exposure within the breast, including transdermal, intraductal and, of our particular interest, transpapillary drug delivery.

Traditional transdermal drug delivery involves application of a patch or gel on the surface of breast tissue. This passive technique achieves similar breast tissue drug concentrations compared to oral treatment, 5-fold reduced plasma concentrations, and extended retention of drug compared to other application sites, thus validating local treatment as a non-toxic treatment alternative (Lee et al., 2014, 2015). However, due to the tight, ordered packing of the stratum corneum, this technique is limited to small, lipophilic compounds that are able to efficiently traverse the skin's innate barrier. Furthermore, this delivery technique is not highly localized within the mammary ductal network. The drug primarily exposes the epidermis and underlying fatty tissue rather than the mammary duct epithelium, which is the origin of a vast majority of breast cancers (American Cancer Society, 2016).

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The first proposed highly localized method was intraductal drug delivery, whereby a small catheter is used to inject a drug solution into mammary duct orifices. Clinical studies confirm the feasibility of repeated identification, cannulation, and injection of the mammary duct orifices, as well as exposure of the entire length of the mammary duct to the injected solution (Love and Barsky, 2004; Love et al., 2013). However, a local anesthetic and a trained surgical professional would be required for administration, leading to questions of practicality for wide-spread adoption, particularly for long-term treatment regimens, and the possibility of self-administration.

A second highly localized strategy, transpapillary drug delivery, offers a non-invasive approach that capitalizes on the mammary ducts to serve as an entry point, conduit, and reservoir. Although previous studies have established the feasibility of permeation through the nipple (Dave et al., 2014; Davison, 2008; Lee et al., 2010), the associated permeation kinetics and cumulative amount of drug permeated vary. While one study contends that equilibrium is reached after 6 h (Lee et al., 2010), others suggest continued permeation for 48 h (Dave et al., 2014, 2016). Furthermore, the total accumulation of molecules and concomitant permeation parameters differ over 200-fold (Alsharif et al., 2017; Dave et al., 2014, 2016). A molecule's physicochemical properties may explain, in part, the extreme kinetic variation, as well as the utilization of transport pathways. Lipophilicity has previously been associated with localization around the mammary duct, while hydrophilic molecules are more likely to diffuse throughout the tissue in an unrestricted manner (Dave et al., 2014), insinuating varied use of transport pathways. However, alternative permeation routes beyond the ductal network have yet to be addressed nor have any permeation pathways been quantified.

The objective of this study was to visualize and quantify the permeation routes and establish the permeation kinetics through the mammary papilla. Two fluorescent dyes, nile red (NR) and sulforhodamine B (SRB), were chosen as model lipophilic and hydrophilic molecules, respectively. These model dyes allowed us to determine the influence of lipophilicity due to their similar molecular weight (MW) (MW = 318.3 and 558.6 Da for NR and SRB, respectively) but differing lipophilicity. A common indicator of lipophilicity is a molecule's octanol-water partition coefficient, or logP (Mannhold et al., 2009). The logP value for NR is 5 (Kuchler et al., 2009) while SRB is = −2 (Amato et al., 2017). These dyes are also relevant models for therapeutic and chemopreventive drugs used clinically in breast cancer treatment and prevention. Tamoxifen (MW = 371.5 Da; LogP = 7.1 (Kim et al., 2016)) and raloxifene (MW = 473.5 Da; LogP = 5.4 (Kim et al., 2016)) are both lipophilic drugs, making NR an appropriate model, while 5-fluororuracil (MW = 130.1 Da; LogP = −0.89 (Kim et al., 2016)) is a hydrophilic drug, similar to SRB. Furthermore, we compared transpapillary to transdermal administration to establish the contribution of mammary ducts to total permeation by assessing differences in transport kinetics between the two routes of delivery.

2. Materials and methods

2.1. Materials

Acetone, Cytoseal60, ethanol, isoamyl alcohol, NR, phosphate-buffered saline (PBS), and SRB were purchased from ThermoFisher. Biosol was purchased from National Diagnostics. All chemicals were used as supplied without further purification. Strips of full thickness porcine abdominal tissue were supplied by a local market and stored at −20 °C prior to use.

2.2. Tissue preparation

Upon use, the nipple or adjacent skin was thawed and dermal fat was mechanically removed. A digital caliper (Traceable Digital Caliper) was used to measure the thickness of each tissue specimen, which was then mounted on a jacketed Franz diffusion cell (surface area 0.64 cm^2; Permegear).

2.3. Fluorescent dye solution preparation

Two fluorescent dyes, NR and SRB, were used as model lipophilic and hydrophilic molecules, respectively. Each dye was used at a 3.14 mM concentration. Because of solubility differences, NR was solubilized in pure ethanol, while SRB was solubilized in a solution of ethanol:PBS at a 1:1 (vol:vol) ratio.

2.4. In vitro diffusion studies

To examine the kinetics of dye diffusion into and through the mammary papillae or skin, the receiver compartment of a Franz diffusion cell was filled with 5 mL of the compatible solvent for each dye (ethanol for NR and 1:1 ethanol:PBS (vol:vol) for SRB) unless otherwise indicated. These solvents were chosen for consistent partitioning from the donor compartment to the tissue and from the tissue to the receiver compartment. This volume was sufficient to ensure direct contact between the tissue and receiver compartment solution. The diffusion cell was maintained at 37 °C with continuous stirring of the receiver compartment. The donor compartment was filled with 300 µl of 3.14 mM NR or SRB in solution and left in contact with the tissue samples for a time period ranging from 30 min to 48 h. At the specified end-point, the donor chamber contents and tissue were removed and the tissue surface was washed three times with 1 mL of the compatible solvent for further processing.

2.5. Tissue Imaging by fluorescence microscopy

Nipples were stored in OCT (Tissue-Tek) and frozen at −80 °C. A cryostat (Leica CM1860) was used to coronally section the nipple from tip to base. At 1-mm intervals, three 10–20 µm sections were collected and mounted on a slide, incubated at 37 °C for 6 h, and sealed using Cytoseal 60. Fluorescence micrographs of the entire tissue section were obtained using a Zeiss Axioplan 2 with a Cy3 filter set (Chroma HQ 41007: HQ 710/75, HQ 515/30, Q 570 LP) at 10x magnification. For each dye, images were taken at the same exposure to minimize variation associated with image data. Images were then compiled using SlideBook 6 Software (Intelligent Imaging Solutions) to create a montage of the entire nipple cross section.

2.6. Generation of fluorescence intensity profile

The plot profile tool in ImageJ software (Schneider et al., 2012) was used to calculate the fluorescence intensity as a function of radial distance across the section. All analysis was performed on images of coronal nipple cross sections from a depth of 1 mm from the tip of the nipple. Each image was subject to eight to ten regions of interest, which were then averaged to generate one intensity profile per tissue section. The region of interest lines were drawn either normal to the tissue edge or normal to the mammary duct towards the inner dermis, generating quantitative fluorescence intensity profiles in the x-y plane.

2.7. In vitro penetration study

Porcine nipples were positioned in the Franz diffusion cell for 30 min to 48 h. Following permeation, the tissue was cut into small pieces with scissors or sectioned on a cryostat (Leica CM1860). When a cryostat was used to cut the tissue, nipples were coronally sectioned in 100 µm increments from tip to base. Tissue sections were collected in 1 mm increments into sample tubes until the tissue was completely sectioned. The weight of sample tubes were recorded pre-and post-addition of tissue to calculate the weight of individual tissue sections. Once the tissue was in small pieces, the dye was extracted from tissue