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Use of the skin sandwich technique to probe the role of the hair follicles in sonophoresis

Omar Sarheed*, Yakov Frum

Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 27 Taylor Street, Glasgow G4 0NR, Scotland, UK

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ABSTRACT

The human skin sandwich technique was used to explore the effect of brief ultrasound exposure on the transfollicular pathway of absorption. Hydrocortisone was used as a model drug. In order to calculate the permeability coefficient of hydrocortisone, its concentration at saturation in the PBS donor solution was determined. Skin samples were prepared by sandwich technique with total hydration of the epidermal and sandwich membranes. The skin was sonicated for 0 s (control), 30 s or 45 s using a pulsed mode (10% duty cycle) with the spatial and temporal average intensity (SATA) of 3.7 W/cm^2. The transducer was then removed and permeation was allowed to proceed for 52 h. Then the percentage follicular contribution was determined. It was determined that without ultrasound, drug entry into follicles accounted for 46% of total penetration. As the duration of sonication increased, the follicular contribution fell to zero even though total transepidermal flux dramatically increased. This is explained by ultrasound exposure causing sloughing off of the uppermost stratum corneum. This permeabilises the continuous surface but at the same time the disturbed cornceocytes will plug hair follicle orifices.

1. Introduction

For many years, researchers investigating topical and transdermal drug delivery have questioned the relative importance of drug absorption through the continuous stratum corneum versus permeation through the hair follicles. Early work indicated that follicular transport was significant only during the initial stage of drug absorption but made only a very small contribution to the flux during the steady-state period (Scheuplein, 1967; Scheuplein et al., 1969). This was believed to be due to follicular openings only making up ~0.1% of the total skin surface area. However, more recent research, using many different approaches and methods has indicated that hair follicles may have a greater role than previously believed (Ogiso et al., 2002; Dokka et al., 2005; Grams et al., 2005; Teichmann et al., 2005, 2006). This new thinking has been associated with the realisation that the follicles really represent invaginated or inward folded areas of the stratum corneum. So the actual surface area for possible absorption is a lot greater than 0.1% (Meidan et al., 2005).

Several animal models have been used over the years to investigate the role of the hair follicles. One simple approach is to simply compare the penetration of a drug through a hairy and hairless rodent species. For example, Hisoire and Bucks (1997) deduced the delivery of retinoic acid to hair follicles by comparing the acid's percutaneous penetration through both hairy and hairless guinea pigs in vitro. A problem with this method is that hairless species of rodents do actually have skin containing underdeveloped follicles and are so not really follicle-free (Meidan, 2010).

Other animal models have included the use of the macaque monkey, the Syrian hamster as well as comparisons between hairy and hairless sites of the guinea pig ear. However, a general problem with all animal models is that it is difficult to show that the barrier properties of any follicle-containing continuous skin membrane are structurally comparable to those of the "control" follicle-free continuous membrane. The problem was addressed by Barry et al. who devised a novel in vitro technique, called the 'skin sandwich' approach (El Maghraby et al., 2001; Barry, 2002; Essa et al., 2002).

In the skin sandwich system, the role of shunts in total percutaneous absorption is determined by comparing drug flux across hydrated epidermal membrane with that through a hydrated 'sandwich' of epidermal membrane with an extra stratum corneum on top. Fig. 1 illustrates such a sandwich. Remembering that follicles typically represent only about 0.1% of the total skin surface area, it follows that there is a negligible probability that hair follicle openings in the two stratum corneum membranes will superimpose. So the additional stratum corneum blocks virtually all available follicular routes. If the shunts route plays no part in the permeation

* Corresponding author at: Department of Pharmaceutics, RAK College of Pharmaceutical Sciences, RAK Medical and Health Sciences University, PO Box 11172, Ras Al Khaimah, United Arab Emirates. Tel.: +971 50 169 8283; fax: +971 72 269 997.
E-mail addresses: sarheed78@gmail.com, omar.sarheed@btinternet.com (O. Sarheed).

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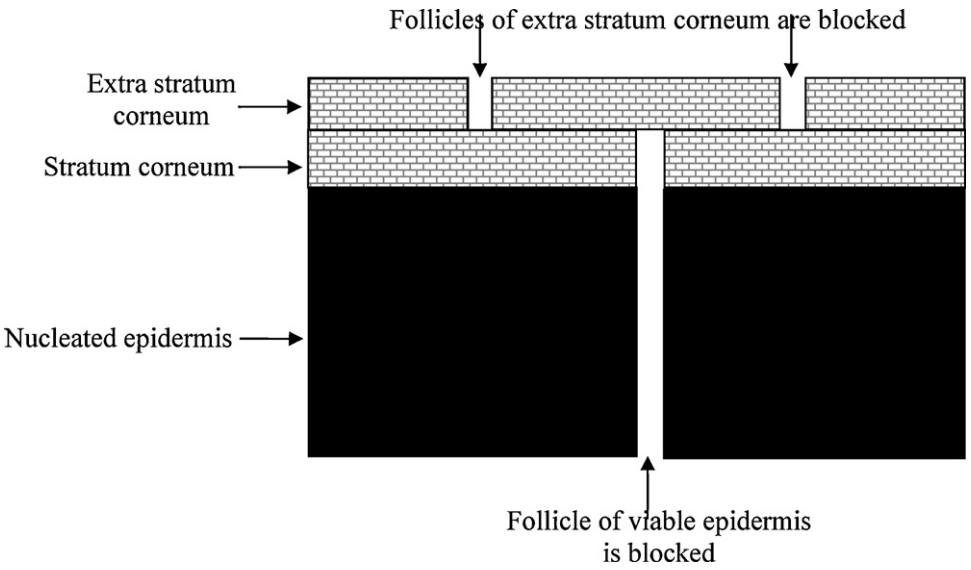

Fig. 1. Scheme demonstrating the basis of the skin sandwich approach. The sandwich consists of an epidermal membrane with an overlying extra stratum corneum. The arrangement effectively blocks the follicular passage of applied solutes.

process then steady state flux through the sandwich is half that of the single membrane. If shunts are responsible for all drug transport then flux through the 'sandwich' is zero. Hence, the magnitude of flux decrease allows quantifiable identification of shunt contribution to total absorption. The shunts are believed to correspond to the hair follicles since the much smaller sweat duct openings close over when the tissue is fully hydrated.

Two further basic points should be considered. Firstly, it has long been established that as long as relatively non-lipophilic drugs ($\log K_o/w < 2.7$) are used, the nucleated epidermis does not contribute significantly to skin barrier properties. As a result, the presence of the nucleated epidermis in the sandwich membrane can be neglected in permeability calculations and it just acts as a mechanical support. Hence, for the purposes of calculations, the thickness of the sandwich is considered to be double the thickness of the single stratum corneum (with the nucleated epidermal membrane). Another important point is that when the skin sandwich system is used, the extra stratum corneum and the lower stratum corneum are extracted from adjacent skin areas on the same donor, thus reducing as far as possible, natural variations in skin barrier properties.

The objective of present study was to use the skin sandwich technique to explore the effects of ultrasound on the transfollicular route of absorption. Hydrocortisone was employed as a model drug. Since initial pilot studies have indicated that epidermis and stratum corneum isolation was very difficult to achieve with pig skin, human skin was used in these studies.

2. Materials and methods

2.1. Materials

Hydrocortisone, bovine pancreatic trypsin (T-4665) and phosphate buffer saline (PBS) tablets (pH 7.4) were all purchased from Sigma–Aldrich (Poole, UK). Tritiated [1,2,6,7-3H]-hydrocortisone (74 Ci/mmol) was obtained from Amersham Biosciences (Little Chalfont, UK). Sodium azide was supplied by Acros Organics (Geel, Belgium). Scintillation fluid (Optiphase HiSafe 3) was supplied by Fisher Scientific (Loughborough, UK). Scintillation vials were purchased from Fisher Packard Instrument Co. (Meriden, CT). Double distilled, de-ionised water was employed throughout.

2.2. Preparation of the hydrocortisone-containing donor solution

The donor solution consisted of hydrocortisone present as a saturated solution in phosphate buffer saline (PBS, pH 7.4). This was made by adding excess 'cold' hydrocortisone to PBS (pH 7.4) and stirring for 48 h at 32 °C. The solutions were subsequently filtered through a 6 μm pore cellulose membrane filter (Whatman Ltd., Brentford, UK). Finally, the 'cold drug' saturated solution was spiked with a tiny amount of tritiated hydrocortisone solution and mixed thoroughly such that each donor solution exhibited an activity of 5 μCi/ml. Although addition of this small volume was technically moving the solute concentration away from saturation, the change was negligibly small given the tiny aliquot volume.

2.3. Determination of hydrocortisone solubility in PBS

In order to be able to calculate the permeability coefficient of hydrocortisone, it is necessary to know its concentration at saturation in the PBS donor solution. The value was determined by using a radioactivity-based method. An aliquot of tritiated hydrocortisone (20 μCi) was added to 10 mg of 'cold' hydrocortisone. This was allowed to evaporate to dryness overnight. Subsequently, 20 ml of ethanol was added to hydrocortisone and thoroughly mixed to provide radiolabelled hydrocortisone solution. Aliquots of increasing volume (representing increasing mass of hydrocortisone) were added to separate scintillation vials, allowed to evaporate overnight, and reconstituted with 100 μl of PBS (pH 7.4). Liquid scintillation counting of this solution yielded standard curves relating disintegrations per minute to total drug mass. To determine saturation solubilities in PBS, remaining solution of hydrocortisone was separately evaporated to dryness overnight and 10 ml PBS (pH 7.4) was added. The solution was stirred and filtered as described above to prepare radiolabelled saturated solution of hydrocortisone. Each saturated solution was then assayed by liquid scintillation counting. Each experiment was performed in triplicate.

2.4. Sourcing of human skin

Full-thickness cadaveric human skin samples were obtained from the National Disease Research Interchange (Philadelphia, PA). The skins were derived from the abdominal sites of elderly