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Development and validation of an alternative disturbed skin model by mechanical abrasion to study drug penetration

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ABSTRACT

Pharmaceuticals and cosmetics for dermal application are usually tested on healthy skin, although the primary permeation barrier, the stratum corneum, is often impaired by skin diseases or small skin lesions, especially on the hands. These skin conditions can considerably influence the permeation of chemicals and drugs. Furthermore, risk assessment for example of nanoparticles should be performed under various skin conditions to reflect the true circumstances. Therefore, an alternative and reproducible method for a high throughput of skin samples with impaired skin barrier was developed and verified by skin permeation studies (25 h) of caffeine, sorbic acid and testosterone compared to healthy (untreated) and tape-stripped skin. Skin barrier disruption was controlled by *TEWL* measurement.

Skin permeation of the three substances was increased in tape-stripped and abraded skin compared to untreated skin due to the reduced barrier integrity. Enhancement of drug uptake was highest for the most hydrophilic substance, caffeine, followed by sorbic acid and lipophilic testosterone. No significant difference in drug uptake studies was observed between the new abrasion method with an aluminum-coated sponge and the tape-stripping method. The obtained results demonstrate that this abrasion method is an alternative way to achieve a disturbed skin barrier for drug and chemical uptake studies.

1. Introduction

Dermatological products are the first choice in the local treatment of skin diseases due to good patient compliance and low systemic exposure. The outermost layer of the skin, the stratum corneum, is formed by corneocytes imbedded in a lipid matrix primarily composed of ceramides, cholesterol and free fatty acids, representing the primary barrier [1]. This effective barrier restricts the penetration and permeation of chemicals as well as active ingredients into the skin. In contrast, skin diseases are often attended by the disorder or even a disruption of the skin barrier, such as a loss of stratum corneum integrity, and thus an increase in transepidermal water loss (TEWL) [2]. *TEWL* values of healthy human skin range between 3.2 and 10.9 g m^{-2} h^{-1} [3–5], whereas an impaired skin barrier increases *TEWL* values by up to 10 times [6–10]. Eczematous skin diseases such as atopic dermatitis, seborrheic eczema, allergic contact eczema and asteatotic eczema, as well as infectious skin diseases, lead to a loss of skin barrier function complemented by an increased *TEWL* [5,6,9,11,12]. Furthermore, ichthyosis and psoriasis, both complex skin disorders, tend to result in approximately 5 times [8] or even 10 times [7] higher *TEWL* values than healthy skin, respectively.

This dysfunction of the skin barrier can be associated with an enhanced percutaneous absorption of the applied agents [13,14] and possible undesirable side effects [15,16]. Thus, it is essential to consider the condition of the skin while developing and testing a new dermatological product. The diversity of dermatological diseases attended by skin barrier impairment and the risk of toxicity during topical treatment emphasizes the importance of an inexpensive and reproducible *in vitro* skin model that simulates and simplifies skin barrier impairment. Substances for the treatment of skin diseases characterized by impaired skin barrier should be tested with healthy skin, the best-case scenario, and with impaired skin, the worst-case scenario, to ensure the safety and effectiveness of dermal products. Therefore, a reliable, low-cost and easy method is needed for *in vivo* and *ex vivo* testing of formulations and substances.

An overview of different *in vivo* and *in vitro* methods to study the penetration and permeation through damaged skin is given by Gattu and Maibach [17,44]. These methods can be divided into mechanical, chemical and biological methods, as well as investigations on clinical diseased skin.

The most common method for simulating a disturbed skin barrier is the tape-stripping method [10,18-20]. The horny cell layers are gradually removed with adhesive tape. The tape-stripping

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method is minimally invasive and can be applied *in vivo* and *ex vivo* for humans as well as for animals (*e.g.*, pigs and rats). The efficacy of the tape-stripping method can be influenced by the anatomical site, the application pressure, the duration of pressure, the removal rate [3] and the type of tape [21]. Furthermore, another difficulty is the inhomogeneous removal of the cell layers due to the elastic network of furrows [22,23], and the required repetitions to achieve an adequate degree of damage are quite time- and cost-intensive using the validated tape-stripping method by the Simonsen and Fullerton [10].

The objective of the present study was to develop an alternative *in vitro* skin model to simulate skin barrier impairment. The following requirements should be fulfilled by this model: simple and quick application, low cost, and good repeatability. Therefore, the stratum corneum was mechanically removed by a sponge with a rough surface, and the permeation of three model drugs, caffeine, sorbic acid and testosterone, which differ in physicochemical properties, such as octanol-water-coefficient (log *P*) and molecular weight (MW), was studied and compared with intact and tape-stripped skin. Caffeine and testosterone are marker compounds recommended by the OECD, and sorbic acid is a preservative frequently used in cosmetics.

2. Materials and methods

2.1. Skin preparation

Untreated porcine ears (domestic pig) were obtained from a local slaughterhouse and immediately transferred to the lab under cool conditions. Porcine ears were washed by rinsing with moderately warm water and wiped with paper towels, and the bristles were carefully shortened by trimming. Full-thickness skin was obtained from the outer side of the porcine auricle [24] and stored at −20 °C for up to 3 months. Porcine skin was chosen due to its similarity to human skin in terms of its morphology and permeability [25] and due to its availability. Prior to skin permeation studies, the thawed porcine full-thickness skin was prepared. Untreated skin (intact skin) and skin with an impaired barrier were used to conduct skin uptake studies. To simulate the impaired barrier, two different methods were compared. Tape-stripped porcine skin was obtained by a successive tape-stripping procedure (D-Squame® tape disks, 22 mm diameter, Cuderm Corp., USA) following Simonsen and Fullerton [10]. The impairment of the skin barrier by abrasion was induced by partial rub-off of the *stratum corneum* using a sponge with an aluminum coating (Spontex® Brillant scourer pad, MAPA GmbH, Germany). Therefore, the sponge was drawn in a smooth motion over the skin surface to reduce the *stratum corneum*. The degree of skin impairment was controlled by measuring continuous transepidermal water loss (TEWL; DermaLAB Cortex Technology, Denmark) during skin preparation. To ensure good reproducibility, the following quality criteria have been defined: initial *TEWL* values for skin samples (skin thickness: 1.40 ± 0.2 mm) have to be within 10 ± 3 g m^{-2} h^{-1}. The final *TEWL* values for tape-stripped and abraded skin were set to 30 ± 2 g m^{-2} h^{-1}, representing serious damage of *stratum corneum* without complete removal (see Section 3.1). Skin samples that did not meet these requirements were discarded. Furthermore, skin biopsies were taken for histological examination (hematoxylin–eosin staining) of the skin impairment.

2.2. Quantification of caffeine, testosterone and sorbic acid

Caffeine, sorbic acid (Caesar & Loretz GmbH, Germany, both) and testosterone (Sigma Aldrich, Germany) were quantified by HPLC (LaChrom Elite® HPLC system, VWR International GmbH, Darmstadt, Germany) and UV detection at 230 nm (caffeine), 255 nm (sorbic acid) or 245 nm (testosterone), respectively. A LiChrospher® 100 RP-18e (5 μm) LiChroCART® 125-4 column (Merck KGaA, Darmstadt, Germany) was used for all test substances. The isocratic mobile phase was 10% acetonitrile (ACN) and 90% phosphate buffer (10 mM, pH 2.6: 0.34 mL/L orthophosphoric acid (85%) and 0.68 g/L $NaH_2PO_4 \cdot H_2O$), delivered with a flow of 1.0 mL/min for caffeine (retention time = 3.2 min, LOD: 5 ng/mL and LOQ: 14 ng/mL), and 30% ACN and 70% phosphate buffer with a flow of 1.2 mL/min for sorbic acid (retention time = 2.3 min, LOD: 9 ng/mL and LOQ: 26 ng/mL); the column temperature 40 °C for each. For testosterone, the mobile phase was a gradient of ACN/phosphate buffer (45:55–85:15 v/v within 10 min followed by a washing procedure) with a flow of 1.0 mL/min. The retention time was 5.1 min, LOD: 23 ng/mL and LOQ: 69 ng/mL; the column temperature was 40 °C.

2.3. Skin permeation and drug uptake studies

Permeation studies of caffeine, sorbic acid and testosterone were performed *in vitro* using the Franz diffusion cell set-up [26], 15 mm in diameter (surface area 1.76 cm^2) and 12 mL acceptor volume (Gauer Glas, Germany). On the day of the experiment, the prepared skin samples (see section Skin Preparation) were mounted into the Franz diffusion cell and allowed to equilibrate for 30 min at 32.5 °C. The acceptor medium was magnetically stirred at 500 rpm. To provide sink conditions, phosphate buffered saline (PBS, pH 7.4) with caffeine and sorbic acid (water solubility at 20 °C: 20 and 1.6 mg/mL, respectively) as well as PBS plus 0.5% Igepal® (Sigma Aldrich, Germany) with testosterone (determined solubility: 93.9 μg/mL) was used as acceptor medium. Then, 1 mL of the donor solution (caffeine and sorbic acid: 1 mg/mL, testosterone: 200 μg/mL with 0.4% ethanol and 2% Igepal®) was applied to the skin surface for 25 h. The donor compartments were sealed with Parafilm® to prevent evaporation of the solution. Aliquots of the acceptor medium (500 μL) were withdrawn repeatedly (every hour from 0 to 10 h and from 21 to 25 h) and replaced with fresh acceptor medium. The samples were analyzed by high-performance liquid chromatography (HPLC). At the end of the experiment, the remaining donor solution was removed by a wash-off procedure and then collected and analyzed by HPLC. Skin samples were wiped with paper towels, snap-frozen, chopped, transferred into a reaction tube and submerged with 1 mL of the extraction solution (caffeine: 10% ACN/90% phosphate buffer; sorbic acid: 30% ACN/70% phosphate buffer; testosterone: 45% ACN/55% phosphate buffer). Skin samples were incubated for 1 h at 50 °C with shaking at 1400 rpm and then centrifuged (5 min and 14,680 rpm, Centrifuge 5424, Eppendorf AG, Germany); the supernatant was subjected to HPLC analysis. The extraction was performed twice. Preliminary studies showed that recovery of the drug from the skin after two extraction steps was $91.7\% \pm 0.9\%$ with caffeine, 94.7 ± 2.5 % with sorbic acid and $82.7 \pm 16.0\%$ with testosterone.

2.4. Data analysis

The cumulative amount of permeated drug, expressed in micrograms per square centimeter (μg/cm^2), is plotted against time (h). The flux, the mass of test substance passing through a unit area of the membrane (1.76 cm^2) per unit of time under steady-state conditions (in μg/cm^2/h) and the relative lag time (abscissa intercept point, h) were calculated from the slope of the graph using an automated approach [27]. Furthermore, the total drug uptake (drug in the skin plus drug in the acceptor medium after 25 h) and the total recovery were determined. Apparent permeability (P_{app}) is presented in Box-and-Whiskers plot (Min to