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The role of hair follicles in the percutaneous absorption of caffeine

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- In recent years, it has been suggested that hair follicles represent important shunt routes into the skin for drugs and chemicals [1–3].
- *In vitro* studies have shown the importance of skin appendages for skin penetration by hydrophilic compounds [4]. Investigation of follicular penetration *in vivo* has been difficult due to the absence of appropriate analytical methods or suitable animal model systems.
- Recently, a new method was described that quantifies follicular penetration *in vivo* by using selective closure of hair follicles [5].
- Caffeine is frequently used in skin penetration experiments as a model for highly water-soluble compounds. Occlusion [6] and skin thickness [7] seem to have little influence on the penetration of caffeine. However, percutaneous absorption rates for caffeine exhibit regional skin differences in humans *in vivo* [1].

WHAT THIS STUDY ADDS

- The results of the present study demonstrate that a fast drug delivery of caffeine occurs through shunt routes. Therefore, hair follicles are considerable weak spots in our protective sheath against penetration into the body by hydrophilic substances.
- We showed that there is a quantitative distinction between follicular penetration and interfollicular diffusion of caffeine *in vivo*.
- These findings are of importance for the development and optimization of topically applied drugs and cosmetics. In addition, such properties must be considered in the development of skin protection measures.

AIMS

The skin and its appendages are our protective shield against the environment and are necessary for the maintenance of homeostasis. Hypotheses concerning the penetration of substances into the skin have assumed diffusion through the lipid domains of the stratum corneum. It is believed that while hair follicles represent a weakness in the shield, they play a subordinate role in the percutaneous penetration processes. Previous investigation of follicular penetration has mostly addressed methodical and technical problems. Our study utilized a selective closure technique of hair follicle orifices *in vivo*, for the comparison of interfollicular and follicular absorption rates of caffeine in humans.

METHODS

Every single hair follicle within a delimited area of skin was blocked with a microdrop of a special varnish-wax-mixture *in vivo*. Caffeine in solution was topically applied and transcutaneous absorption into the blood was measured by a new surface ionization mass spectrometry (SI/MS) technique, which enabled a clear distinction to be made between interfollicular and follicular penetration of a topically applied substance.

RESULTS

Caffeine (3.75 ng ml^{-1}) was detected in blood samples, 5 min after topical application, when the follicles remained open. When the follicles were blocked, caffeine was detectable after 20 min (2.45 ng ml^{-1}). Highest values (11.75 ng caffeine ml^{-1}) were found 1 h after application when the follicles were open.

CONCLUSIONS

Our findings demonstrate that hair follicles are considerable weak spots in our protective sheath against certain hydrophilic drugs and may allow a fast delivery of topically applied substances.

Introduction

Our skin and its specialized outermost layer, the stratum corneum, protects us against the penetration of chemicals and the invasion of micro-organisms from the environment and it also prevents an extensive loss of water from the body. The skin barrier has been described as a 'bricks-and-mortar-model', similar to a wall, the corneocytes acting as bricks, surrounded by specialized lipid layers as the mortar [2, 8]. Until recently, skin appendages were believed to play a subordinate role in skin barrier function, although it was consistently assumed that hair follicles and sweat glands could provide shunt routes through the stratum corneum [9–11]. Moreover, the orifices of appendages were estimated to represent not more than 0.1% of the total skin surface. These estimates were recently corrected through measuring follicular orifice size and distribution of vellus hair follicles in different skin areas. Out of seven skin areas examined, forehead skin showed the highest follicular density compared with the extremities (calf region and forearm). It was found that the surface area of the follicular epithelium can be seen as a considerable enlargement of the skin surface, with variability in contribution depending on the skin area, follicular size and density [12]. The follicular epithelium shows morphological differences compared with the interfollicular epithelium [13] and can be seen as a less resistant component in the skin barrier. Rougier *et al.* [1] found differences in skin permeability for benzoic acid, benzoic acid sodium salt, caffeine and acetylsalicylic acid in different body areas. The lowest permeability was found on the forearm in contrast to the forehead, which showed the highest absorption rates. Shunt routes were assumed to play a role in these findings although they were not quantitatively investigated for their contribution [1]. Essa *et al.* [4] demonstrated the significance of shunt routes for the absorption of mannitol *in vitro,* by using an epidermis and stratum corneum sandwich model.

Caffeine, a naturally occurring purine based alkaloid, is frequently used as a hydrophilic model substance in skin penetration experiments (log *P* –0.01) [6, 7, 11, 14–21]. Caffeine penetration was shown to be unchanged by occlusion [6] and skin thickness [7]. Recently, it was asserted that topically applied caffeine reduces carcinogenesis up to 70% in UVB-radiated mice [22, 23]. Moreover, current studies presume a stimulating effect on hair growth through topically applied caffeine, which makes caffeine even more interesting for the investigation of follicular penetration [24].

In our present study, we used a method, which allowed us to block selectively follicular orifices to evaluate the transport of caffeine though the skin via hair follicles relative to the absorption through the interfollicular epidermis.

Methods

Volunteers

The study was carried out on six healthy Caucasian male volunteers aged 26–39 years with normal body mass indices (between 21 and 24) and pronounced terminal hair on the chest. The study was approved by the Ethics Committee of the Charité – Universitätsmedizin Berlin, Germany. All volunteers had given their written consent prior to the study.

Model substance

A 2.5% caffeine containing formulation was applied (caffeine (Sigma-Aldrich Chemie, Steinheim, Germany) 2.5% w/v, ethanol (Uvasol, Merck, Darmstadt, Germany) 30%, and propylene glycol (Fluka, Sigma-Aldrich, Buchs, Switzerland) 70%.

Study protocol

Caffeine has a half-life of 3.1–6.7 h in human blood [25]. Therefore the volunteers had to adhere to a strict caffeine free diet, 2 days before, and during, the entire experiment. Volunteers abstained from coffee (including decaffeinated coffee), black, green and white tea and tea products of any kind, chocolate, energy drinks, and other carbonated drinks containing caffeine. The volunteers were asked to enter the test laboratory 1 h before the experiment started. Test conditions were kept constant with a room temperature of 22°C and 50% humidity.

Baseline blood samples were taken before topical application of the caffeine formulation. Hair shafts were clipped to a length of 0.5 mm in an application area of 25 cm^2 in size on the chest of the volunteers. Hair follicle density averaged between 20 and 32 follicles cm^{-2}. Two different set ups were performed at intervals of 7 days. Set up 1 was used as control group: One micro drop of varnish-wax-mixture was placed beside each hair follicle orifice using a 1 ml syringe with a blunt 30 gauge needle. The sharp tip of the needle had been cut off with a metal cutter. Using a syringe and a spatula for distribution over the test area, 2 mg cm^{-2} of the 2.5% caffeine containing solution was applied to the test area (10 µg caffeine cm^{-2}). The relatively small amount of the viscous solution stayed in the test area and had a shiny appearance which enabled easy location identification. The formulation was allowed to evaporate for 8 h. During these 8 h, the volunteer was not allowed to touch or cover the area. After 8 h, the volunteers were allowed to get dressed again. The volunteers were asked not to shower or bath for a duration of 72 h. Blood samples were taken 5, 10, 20 and 30 min as well as 1 h, 2 h, 5 h, 8 h, 24 h and 72 h after the topical caffeine application.

Set up 2 was conducted after a further 3 days of caffeine free diet. The experiment was repeated on exactly the same skin areas of the same volunteers under the same conditions. Again, caffeine blood concentrations were

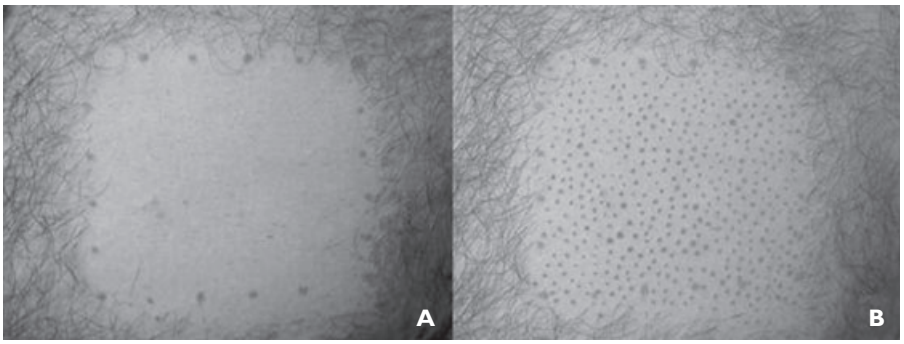

Figure 1
Follicular blocking. Test area (25 cm^2) before (A) and after (B) follicular block

tested before application of the caffeine formulation. Subsequently, hair shafts were clipped again and each hair follicle orifice was blocked with a microdrop of the varnish-wax-mixture [5] prior to the caffeine solution being applied. Again, blood samples were taken at equivalent time points up to 72 h post application.

Digital image analysis showed that the microdrops of the varnish-wax-mixture in both set ups covered approximately 8% of the epidermis. Figure 1a and b show the test area of volunteer 6 before and after the application of the varnish-wax-mixture in set up 2 (with follicular block).

Caffeine was extracted from the serum samples with dichloromethane via acidic extraction [26].

An aliquot (200 µl) from each serum sample was measured by a new surface ionization mass spectrometry (SI/MS) technique, developed at the Arifov Institute of Electronics of the Uzbek Academy of Sciences, Taschkent, Uztbekistan [27]. This technique was developed for the detection of minute amounts of drugs. The measuring system is based on the highly efficient selective ionization of caffeine molecules, adsorbed on an emitter surface. By heating this emitter, the molecules became selectively ionized [28]. The lower detection limit of the SI/MS method is 1 ng caffeine ml^{-1}.

Statistical analysis

For statistical analysis, we utilized the Wilcoxon test and SAS® software (SAS®, Statistical Analysis System Institute Inc., North Carolina, USA).

Results

In set up 1, when hair follicles were left open, mean values of 3.75 ng caffeine ml^{-1} were detected in the blood samples taken 5 min after topical application of the caffeine formulation (Figure 2). There was relatively small interindividual deviation in the results as demonstrated by low standard deviation values. In set up 2, when hair follicle orifices were blocked, caffeine could not be detected in the blood samples until 20 min after application. The caffeine concentration averaged 2.45 ng ml^{-1} after 20 min with a SD of 1.26 ng ml^{-1} when follicles were blocked. In contrast, the blood concentrations at 20 min post application were significantly higher (7.57 ng ml^{-1}) when the follicles were open (set up 1). Highest values with an average of 11.75 ng caffeine ml^{-1} were found in set up 1, 1 h after application and highest values in set up 2 were reached after 2 h with an average of 6.65 ng ml^{-1} (Figure 2). In general, caffeine blood concentrations were lower when the follicular pathway was blocked. Caffeine absorption took at least four times longer in set up 2, when the follicles were closed for penetration. No caffeine could be detected 72 h after application in both set ups.

Discussion

The present study shows the absorption of topically applied caffeine *in vivo* on exactly the same skin area with and without the influence of hair follicles. A significantly faster absorption of caffeine was detected when the hair follicle orifices were open. The artificial blocking of the hair follicle orifices ensured they were excluded as possible drug penetration routes. As a consequence of this, caffeine should only pass through the interfollicular epidermis and its lipid domains and possibly through the sweat glands. With the follicular orifices blocked, caffeine penetration of the skin took much longer and the maximum measured caffeine blood concentrations were generally lower.

Our results show that interfollicular and follicular penetration is a simultaneous process. The relation between both pathways is kinetically controlled, which is directly after application of the caffeine formulation, staggered for the benefit of the follicular pathway. The kinetics of both penetration processes are likely to be dependent on the nature of substance and vehicle employed. However, our results clearly demonstrate that hair follicles can be significant access routes for chemical compounds and they should not be ignored in the development and optimiza-

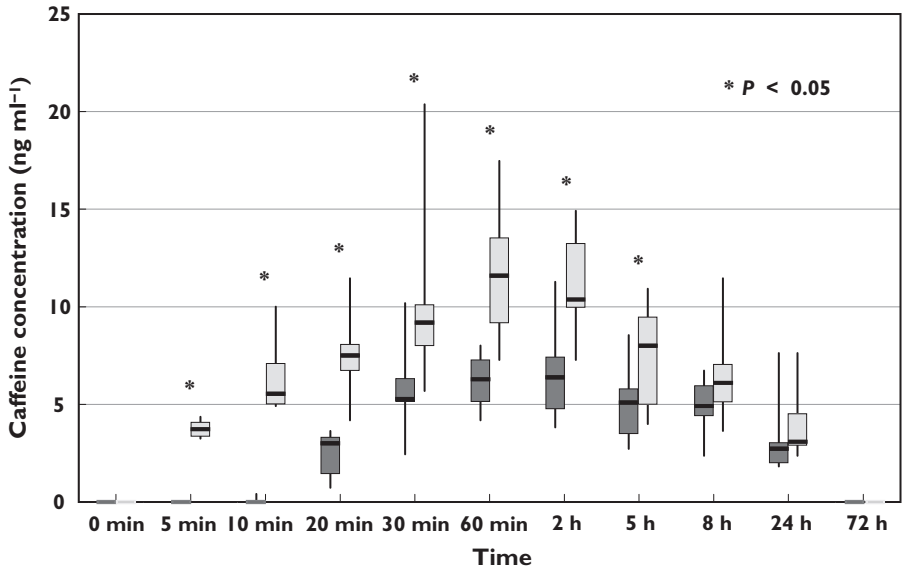

Figure 2

Caffeine analysis with the surface ionization method. Caffeine blood concentrations after topical application with and without artificial block of the hair follicles (closed follicles, (■); open follicles, (□)). The box and whisker plots show the median (thick horizontal line) and the 25% and 75% percentiles

tion of topically applied drugs and cosmetics. Also, for the development of skin protection measures, such as barrier creams or decontamination compounds, the rate of follicular drug delivery has to be taken into account.

In preceding experiments, it was shown that hair follicles can act as a relevant reservoir for topically applied substances [12] and that nanoparticles and liposomes at a size of 300–750 nm preferentially penetrate into the hair follicles [29, 30]. The present study is limited to only one hydrophilic model compound, caffeine. Randomized controlled studies will be necessary to evaluate the role of hair follicles in the cutaneous penetration of other substances with different physical properties. However, the data suggest that hair follicles may provide a significant access route for certain drugs and compounds. Knowledge of quantitative drug delivery through hair follicles could be used for the optimization of the treatment of hair follicle related skin disorders such as androgenetic alopecia or acne. More extensive research on follicular penetration will lead to a better understanding of skin barrier function and will be necessary for an optimization of follicular drug delivery.

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