

Follicular Penetration of Topically Applied Caffeine via a Shampoo Formulation

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Key Words

Surface ionization • Follicular penetration of caffeine

Abstract

Aims: Follicular drug delivery is the prerequisite for an effective treatment of androgenetic alopecia or other reasons of premature hair loss. **Methods:** The follicular penetration of caffeine, applied topically in a shampoo formulation for 2 min, was measured with highly sensitive surface ionization in combination with mass spectroscopy, a selective method for the detection of very small quantities of transcutaneously absorbed substances in the blood. An experimental protocol, developed to selectively block the follicular pathway within the test area, was used. Based on this principle, a clear distinction between interfollicular and follicular penetration of topically applied caffeine was feasible. **Results:** After 2 min, caffeine penetrated via the hair follicles and stratum corneum. **Conclusion:** It was found that the penetration via hair follicles was faster and higher compared with the interfollicular route and that hair follicles are the only pathway for fast caffeine absorption during the first 20 min after application.

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Introduction

Topical delivery of active compounds to different compartments of the skin and its appendages is the prerequisite for the efficient treatment of skin diseases. Different methods are available for the qualitative and quantitative measurement of drugs in the skin, for example tape stripping for the drug distribution in the stratum corneum [1, 2], microdialysis for the measurement of the concentration in the dermis [3], qualitative monitoring of a biological effect such as blanching, redness or general changes in blood flow [4, 5] and measurements of the drug levels in the blood. The absorption of topically applied compounds and their levels in the blood vary considerably in the different regions of the human body [6, 7]. The density of hair follicles significantly contributes to this effect by an increase in surface area and a disruption of the epidermal barrier towards the lower parts of the hair follicle. Hereby, the human hair follicle serves not only as a reservoir, but also as a major entry point for topically applied compounds [8, 9]. However, the determination of the penetration and the absorption of drugs into and through the hair follicles has involved methodical and technical problems in the past.

The aim of the present study was to show the follicular absorption of caffeine, applied in a shampoo formulation.

Therefore, a new method was used which allows a selective block of the follicular orifice, and therefore, enables us to distinguish between transepidermal and transfollicular penetration [10].

Caffeine, a naturally occurring purine-based alkaloid, is known for good penetration properties [7]. Current studies presume a stimulating effect on hair growth through topically applied caffeine. Recently, it was asserted that topically applied caffeine reduces carcinogenesis up to 70% in ultraviolet-B-radiated mice [11, 12]. Caffeine is considered to have great potential as a natural antioxidant [13] and is assumed to be beneficial to various aspects of health [14, 15].

Material and Methods

The study was carried out on 6 healthy male Caucasian volunteers between 28 and 38 years of age, of neither seborrhic nor sebostatic skin type, with no history of severe diseases and no sun exposure at least 4 weeks before the experiment.

All volunteers had intense terminal hair growth with a density ranging from 22 to 35/cm² in the chest region. The body mass indices ranged between 21 and 24. The structure of the hair is comparable in men with beard hair or scalp hair. However, the density is approximately 10 times higher on the scalp compared with the chest [16, 17]. Due to technical reasons of the experimental protocol, the chest region was chosen.

Two absorption experiments were carried out in each volunteer at intervals of 1 week. A shampoo formulation with a caffeine content of 1% (Coffein Shampoo C1®; Alpecin, Dr. Kurt Wolff GmbH & Co. KG, Bielefeld, Germany) was applied to a test area on the chest. In the first experiment, the follicle orifices remained open in the test area, whereas in the second absorption experiment, follicles were closed with a special varnish-wax mixture [10].

Caffeine has a half-life of 3.1–6.7 h in human blood [18]; therefore, the volunteers had to adhere to a strict caffeine-free diet 2 days before and during the entire experiment.

The volunteers were positioned to lie on their back. The test area was surrounded by a barrier material to avoid lateral spreading of the formulation and the washing solution. Caffeine levels in the blood were tested before topical application of the caffeine formulation. Hair shafts were clipped to a length of 0.5 mm in an application area of 25 cm² in size. Hair follicle density averaged between 22 and 35 follicles/cm².

In experiment 1, follicles remained open, but to rule out an influence of the closing material on the absorption process, one micro drop of a special varnish-wax mixture was placed beside each hair follicle orifice. We applied 2 mg/cm² of the 1% caffeine-containing shampoo formulation to the test area for 2 min. After 2 min, the shampoo was removed by a standardized washing procedure. Blood samples were taken 5, 10, 20 and 30 min as well as 1, 2, 5, 8, 24 and 72 h after the topical caffeine application.

After 1 further week of caffeine-free diet, the experiment was repeated on exactly the same skin areas. Subsequently, hair shafts were clipped again and each hair follicle orifice was blocked with

a micro drop of the varnish-wax mixture prior to the shampoo formulation being applied for 2 min. Again, blood samples were taken at different points in time up to 72 h after application.

Digital image analysis showed that the micro drops of the varnish-wax mixture in both set-ups closed approximately 8% of the epidermis.

Caffeine from the blood samples was extracted from the serum with dichloromethane via acidic extraction. Each extract was analyzed by a highly sensitive technique, a mass spectrometer with a surface ionization system (SI/MS) developed at the Arifov Institute of Electronics of the Uzbek Academy of Sciences, Tashkent, Uzbekistan [19]. The detection limit of the SI/MS method for caffeine was 1 ng/ml.

Results

The results of the caffeine analysis of the blood samples showed a concentration of 6.3 ng/ml 5 min after application when transfollicular penetration was possible. In contrast, relevant concentrations of caffeine appeared in the blood only after 20–30 min in cases when follicles were closed. Figure 1 shows the results of the caffeine analysis with the surface ionization method. Data are expressed as means and standard deviations, with $p \leq 0.05$ considered significant. For statistical analysis, we utilized the Wilcoxon test and SAS® software (SAS; Statistical Analysis System Institute Inc., Cary, N.C., USA).

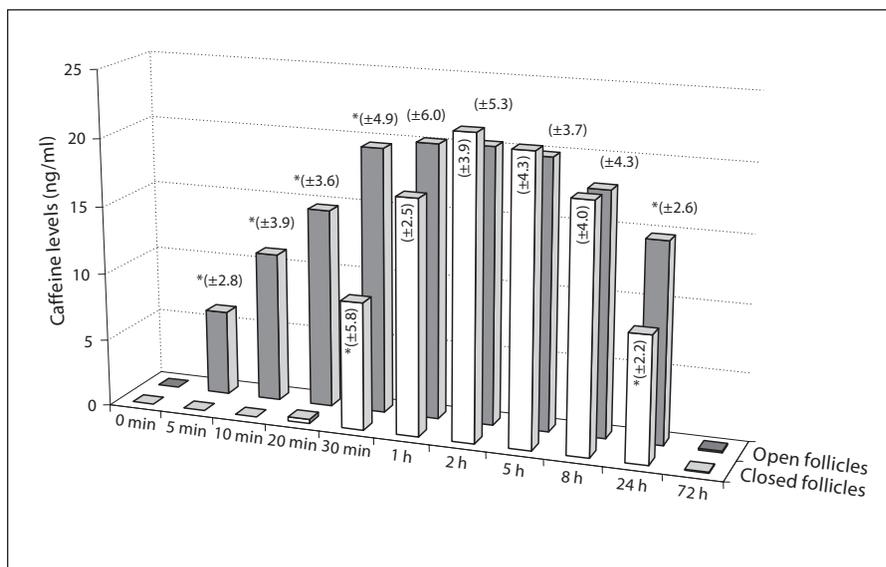
Highest values at an average of 20.4 (experiment 1) and 22.2 ng caffeine/ml (experiment 2) were found 2 h after application. A relatively low standard deviation expresses small interindividual deviations. No caffeine could be detected 72 h after application in either set-up.

Discussion

The results clearly show that after a 2-min application of caffeine in a shampoo formulation and a standardized washing procedure, caffeine penetrated into the stratum corneum and into the hair follicles. Absorption through the hair follicles and relevant caffeine levels in the blood were already found 5 min after topical application, whereas comparable levels could only be obtained after 20 min when caffeine permeated only through the stratum corneum of the interfollicular epidermis. Relevant levels of caffeine in the blood were detected 20–30 min after application in cases when follicles were blocked.

The present study shows that a 2-min application of the caffeine shampoo is sufficient for being able to detect caffeine levels in the blood after a transcutaneous and transfollicular caffeine penetration. Moreover, it can be

Fig. 1. Caffeine levels in the blood after topical application with and without artificial blocking of the hair follicles. Data are expressed as means and standard deviations, with * $p \leq 0.05$ considered significant.



assumed that a caffeine reservoir is formed in the skin for at least 8 h and caffeine is constantly released. Relatively high levels of caffeine in the blood were still found after 24 h.

The results indicate that interfollicular and follicular penetrations are parallel processes. The relation between both pathways is kinetically controlled, which occurs directly after application of the caffeine formulation, staggered for the benefit of the follicular pathway.

The reason for the improved penetration through the hair follicles can most likely be found in the ultrastructure of the follicular epithelium and in the special environment of the follicular infundibulum. The hair follicle epithelium shows an epidermal differentiation in the infundibulum. The epithelium of the uppermost parts shows no difference to the interfollicular epidermis; in the lower parts of the infundibular epithelium, corneocytes are smaller and appear crumbly. This part of the follicular epithelium can be seen as an incomplete barrier for topically applied substances [20]. In general, caffeine is known for its good penetration through the skin. In principle, caffeine can penetrate through all parts of the follicular epithelium. As mentioned above, lower parts of the follicular epithelium are seen as a major entry point for topically applied substances.

For a physiologic effect of caffeine on the hair cycle, it can be assumed that caffeine has to reach the follicular papilla and the bulge region of the hair follicle. On the one hand, it is imaginable that caffeine reaches the regulatory cells of the hair follicle via a direct penetration into

the follicular epithelium and, on the other hand, via the microcapillary system which surrounds the hair follicles. The investigation of the regulatory effect of caffeine on the human hair cycle is under examination in current *in vivo* studies.

Conclusion

The *in vivo* analysis of the penetration of caffeine contained in a shampoo via the follicular penetration pathway into human blood was demonstrated by selective blocking of the hair follicles. Using the highly sensitive surface ionization method in combination with mass spectroscopy, it became possible to detect a very small quantity of transcutaneously absorbed caffeine in the blood. The penetration via the hair follicles is faster in comparison with the interfollicular route. The findings demonstrated that the hair follicles might present an efficient penetration pathway for topically applied substances.

Acknowledgements

We would like to thank the Foundation 'Skin Physiology' of the Donor Association for German Science and Humanities for financial support.

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