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Research paper

Follicular and percutaneous penetration pathways of topically applied minoxidil foam

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ABSTRACT

In the past, it was assumed that the intercellular route was the only relevant penetration pathway for topically applied substances. Recent results on follicular penetration emphasize that the hair follicles represent a highly relevant and efficient penetration pathway and reservoir for topically applied substances. This study investigates a selective closure technique of hair follicle orifices *in vivo* assessing interfollicular and follicular absorption rates of topical minoxidil foam in humans. In delimited skin area, single hair orifices or interfollicular skin were blocked with a microdrop of special varnish–wax-mixture *in vivo*. Minoxidil foam (5%) was topically applied, and transcutaneous absorption was measured by a new surface ionization mass spectrometry technique in serum. Different settings (open, closed or none of both) enabled to clearly distinguish between interfollicular and follicular penetration of the topically applied minoxidil foam. Five minutes after topical application, minoxidil was detected in blood samples when follicles remained open, whereas with closed follicles 30 min were needed. Highest levels were found first when both pathways were open, followed by open follicles and subsequently by closed follicles. These results demonstrate the high importance of the follicular penetration pathway. Hair follicles are surrounded by a dense network of blood capillaries and dendritic cells and have stem cells in their immediate vicinity, making them ideal targets for drug delivery.

1. Introduction

The skin barrier for topically applied substances, such as medical or cosmetic products, is formed by the outermost skin layer, the stratum corneum [1,2]. It consists of cornified cells, the corneocytes, which are surrounded by lipids, previously described as bricks- and mortar-model[1]. Molecules have to overcome this horizontal, multilayer stratum corneum in order to penetrate into or through the skin. Vertical structures like hair follicles and especially their lower, infrainfundibular part, lack a mature stratum corneum. They are an important target structure for molecules to penetrate through the skin, being surrounded by a close network of capillaries and dendritic cells [3]. At present, three penetration pathways have been theoretically proposed: the intercellular penetration pathway through the lipid layers surrounding the corneocytes [4], the follicular penetration pathway into the hair follicles and the transcellular penetration pathway [5]. So far, no evidence exists in support of the transcellular penetration pathway. In previous studies, it was assumed that the intercellular route was the only relevant penetration pathway [6,7]. Recently, the hair follicles have increasingly been recognized as an important pathway for percutaneous penetration, although the role of the follicles has still not been clarified in detail [8–10]. The follicular penetration plays a major role in skin penetration pathways and kinetics of topically applied molecules [11–15,5]. More specifically, the relevance of hair follicles in these processes appears to depend on distribution of hair follicle density on different body sites [16,17]. Hereby, the human hair follicle serves not only as a reservoir, but also as a major entry point for topically applied compounds [9,18]. In spite of recent investigations substantiating the importance of hair follicles as target and reservoir structure for topical substances, few data exist on the penetration of molecules through the hair follicle into the blood [19,20]. In the present study, a previously established method was used, which allowed to block the follicular orifices selectively to evaluate the penetration of the model therapeutic agent minoxidil, through the skin via hair follicles relative to the absorption through the interfollicular epidermis [20].

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Notwithstanding the fact that the artificial closing of the hair follicles and the surface ionization mass spectrometry are both sophisticated and exacting methods, their combined application permits for the first time to immediately distinct follicular and intercellular penetration in the human skin *in vivo*.

2. Methods

2.1. Volunteers

After having obtained written informed consent, seven healthy Caucasian male volunteers aged between 22 and 29 years with normal body mass index (range 21–24] and pronounced terminal hair on the chest were included in the study. The volunteers were not allowed to take oral and/or topical drugs or compounds containing minoxidil 4 weeks prior and during the entire study phase. One of the volunteers dropped out failing to appear for follow-up. The study had been approved by the ethics committee at the Charité – Universitätsmedizin Berlin, Germany.

2.2. Model substance

Rogaine® 5% foam formulation (Pfizer Consumer Healthcare, Morris Plains, New Jersey, USA) was applied; as active ingredient: minoxidil 5%. Inactive ingredients: ethanol SD40B, butylated hydroxytoluene NF, purified water USP, lactic acid (90%) USP/NF, glycerin USP/NF, citric acid USP/NF, fragrance, cetyl alcohol NF, stearyl alcohol NF, polysorbate 60 NF, propellant P75.

2.3. Study protocol

The chest and the occipital region of the scalp were chosen as test areas, with a test area of 5 × 5 cm (25 cm^2). Hair shafts were clipped to a length of 0.5 mm in each area to be treated on the chest and occipital scalp of the volunteers. The ambient conditions during the course of the study were monitored constantly providing a room temperature of 22 °C and 50% humidity.

Baseline blood samples were taken before topical application of minoxidil foam. Three series were scheduled, at intervals of 10 days, and 0.007 g (+/− 0.002) of minoxidil foam was applied to the test areas, respectively. Each session was performed at the same pharmacokinetic timing; blood samples were taken 5, 10, 20 and 30 min as well as 1, 2, 4, 8, 24, 48 and 72 h after the topical minoxidil application. To avoid the product under study from spreading, each test area was delineated with window color. At setting 1, the application of the study product was done on occipital scalp (control group), in which both penetration pathways, transfollicular and interfollicular, were left open. The formulation was allowed to evaporate for 8 h. During these 8 h, the volunteer was not allowed to touch or cover the area. The volunteers were asked not to shower or bath for a duration of 72 h. At setting 2, each hair follicle orifice was blocked with a microdrop varnish-wax-mixture using a 1-ml syringe with a blunt 30 gauge needle prior to the minoxidil foam being applied. Therefore, the interfollicular (percutaneous) pathway was open. At setting 3, one microdrop of the varnish–wax-mixture was placed beside each hair follicle orifice. Thus, the transfollicular pathway was left open (Fig. 1).

Digital image analysis showed that the microdrops of the varnish-wax-mixture in both set ups (2 and 3) covered approximately 8% of the epidermis.

Minoxidil was extracted from the serum samples with dichlormethane via acidic extraction [21]. 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, Tashkent, Uzbekistan [22]. This technique was developed for the detection of minute amounts of drugs. The measuring system is based on highly selective detection of minoxidil molecules, adsorbed on an emitter surface. By heating this emitter, the molecules became selectively ionized [23].

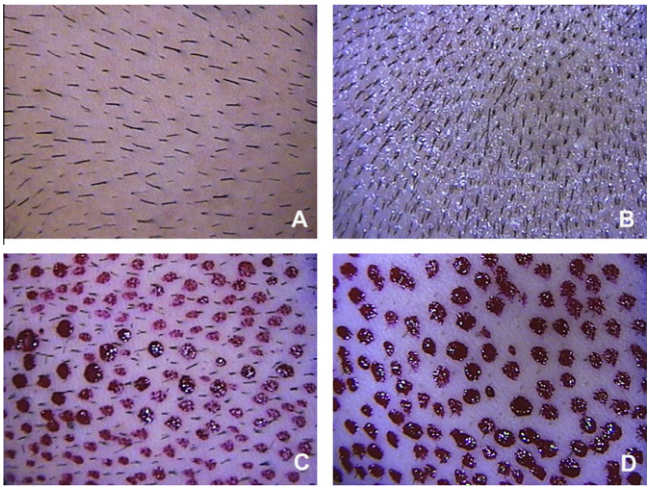

Fig. 1. Test areas stubbly shaved on the chest (A) and occipital scalp (B, control). Red varnish-wax-mixture is used between follicular openings in order to study follicular penetration (C) or to close follicular orifices in order to study interfollicular penetration (D).

2.4. Statistical analysis

For statistical analysis, we used the Wilcoxon test, Friedman-Test SPSS 16.0® Software (Statistical Package for the Social Sciences Inc., Chicago, Illinois, USA).

3. Results

The hair follicle density on the chest area ranged between 36 and 85 follicles per cm^2 (mean = 66) and on the occipital scalp between 123 and 354 follicles per cm^2 (mean = 239).

The analysis of all three settings, i.e., the penetration pathways in all six volunteers is shown in Fig. 2. Blood levels of minoxidil were detected 5 min. after topical application of the minoxidil foam when follicles were left open and when both penetration pathways were possible. In the setting of blocked hair follicles, minoxidil could not be detected in the blood until 30 min after topical application. The minoxidil blood levels of transfollicularly penetrated minoxidil were significantly earlier ($p = 0.027$) detected than in the interfollicular (percutaneous) penetration model. A significantly earlier detection of minoxidil was also observed after application of minoxidil foam onto the control area ($p = 0.046$) compared to the interfollicular (percutaneous) penetration model (Fig. 2). The maximum values of the minoxidil blood concentration tended to be higher for the transfollicular pathway compared to the interfollicular and control pathway based on the mean curve progression, but this observation was not statistically significant ($p > 0.05$). The mean minoxidil blood concentration was higher when comparing the transfollicular pathway to control or interfollicular pathways ($p = 0.046$).

In general, minoxidil blood concentrations were detected earlier when the follicular pathway was not blocked. The minoxidil absorption took at least twelve times longer in setting 2 (closed follicles). No minoxidil blood levels were detected 72 h after application in the three settings.

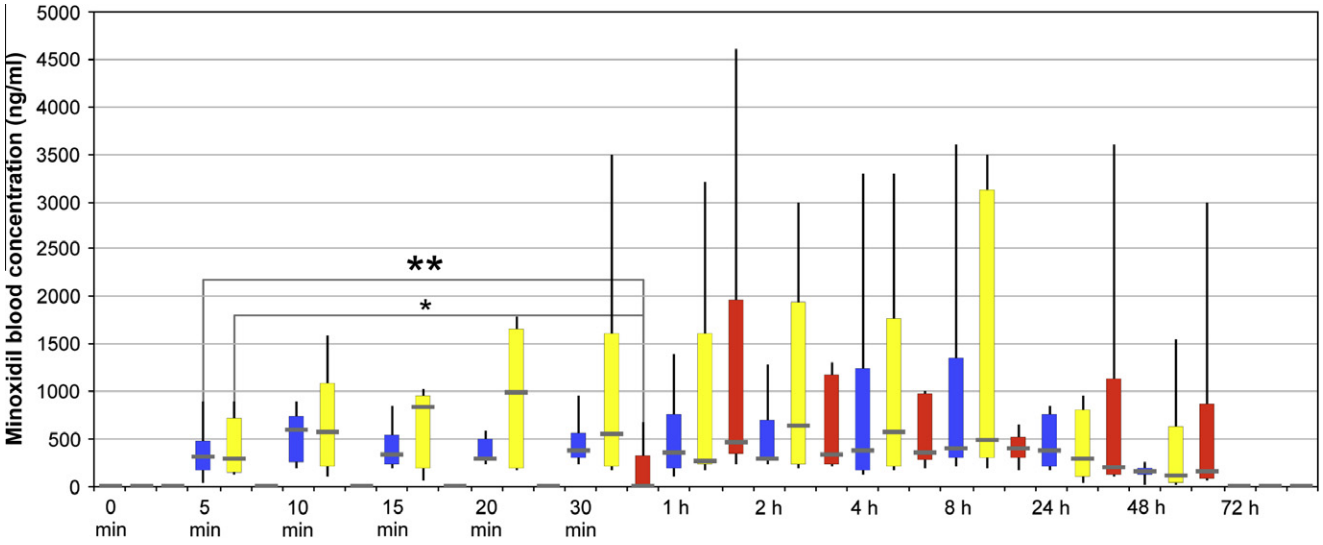

Fig. 2. Minoxidil level analysis using the surface ionization method. Minoxidil blood concentration and its metabolites were significantly earlier detected in the transfollicular ($^{*}p < 0.027$, ■) pathway and in the control ($^{**}p < 0.046$, ■) compared to the interfollicular (percutaneous, ■) pathway.

4. Discussion

The present study demonstrates the absorption of topically applied minoxidil foam *in vivo* on exactly the same skin area with and without the participation of hair follicles. A faster absorption of minoxidil was detected when the hair follicle orifices were open. The artificial blocking of the hair follicle orifices ensured their exclusion as possible drug penetration routes. The minoxidil penetration through the intercellular route took much longer and the maximum of the measured minoxidil blood concentrations tended to be generally lower, when the follicular orifices were blocked. The follicular penetration seems to be a time-dependent and fast process compared to the intercellular pathway, as previously shown with caffeine in a shampoo formulation [20]. In addition, our results show that interfollicular and follicular penetrations are simultaneous processes. The relation of all pathways is kinetically controlled, which is directly after application of the minoxidil foam, staggered for the benefit of follicular pathway. Hair follicles are connected with a network of blood capillaries allowing topically applied molecules to penetrate to the tissue surrounding the follicle and reach the blood circulation, thereby avoiding the stratum corneum barrier [11]. It was recently shown that deposition of minoxidil into appendages and the stratum corneum are similar [19]. Our results demonstrate that hair follicles contribute to the penetration of minoxidil into the blood circulation and support their importance in drug delivery. Transport by the appendageal route is likely to be a key determinant of hair growth promotion by minoxidil [19]. It seems that hair follicles, as well as sweat glands and microlesions in the interfollicular horny layer, are an ideal target for drug delivery and may represent an alternative to the intercellular route of skin permeation. Determination of the penetration into and the permeation through the hair follicles is possible, whereas permeation through the sweat glands and microlesions is still difficult to investigate and very rarely studied. The hair follicles can serve not only as a major entry point but also as a reservoir for dermally applied substances [8,9,13,24]. The present study is limited to the composition of the minoxidil foam. Randomized controlled studies will be necessary to evaluate the role of the hair follicles in the cutaneous penetration of other substances with different physical properties. For future studies, a larger population is required in order to investigate a possible higher absorption of minoxidil through the transfollicular pathway. Knowledge of quantitative drug delivery via the hair follicles could be used for 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 the skin barrier function and will be necessary for an optimization of follicular drug delivery.

Financial disclosure and conflict of interest

Ulrike Blume-Peytavi is consultant to Johnson & Johnson GmbH. Natalie Garcia Bartels has been consultant to Pfizer Pharma GmbH.

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