Topical Formulations Containing Finasteride. Part II: Determination of Finasteride Penetration into Hair Follicles using the Differential Stripping Technique

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ABSTRACT: The differential stripping technique consists of a tape-stripping phase followed by a cyanoacrylate biopsy. This technique not only allows the quantification of drug retained in the stratum corneum (SC) and in the hair follicles but also differentiates transepidermal from transfollicular penetration. Our study aimed at both validating the differential stripping procedure on hairless rat skin and assessing the role of the hair follicle in the cutaneous penetration of finasteride (FNS) after application of two experimental formulations for 6 or 24 h: P-08–016, a hydroxypropyl chitosan (HPCH)-based formulation and P-10–008, an anhydrous formulation devoid of HPCH. Microscopic and histological evaluation showed that after 15 tape strips both the SC and the viable epidermis were completely removed. A subsequent cyanoacrylate skin surface biopsy led to the removal of the infundibula content. The largest amounts of FNS were found in the epidermis and in the appendages after application of P-08–016, regardless of the time from application. In contrast, smaller and statistically significant amounts of FNS were recovered with P-10–008 6 h after application, compared with that at 24 h. In conclusion, the differential stripping technique allowed determination of the amount of FNS localized in different skin districts, focusing particularly on the follicular contribution. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:2323–2329, 2014
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INTRODUCTION

During the last decades, many studies have been performed to determine the penetration pathways of topically applied substances. In particular, the lipid portion of the stratum corneum (SC) was considered as the major pathway, whereas the skin appendages were presumed to play a subordinate role representing not more than 0.1% of the total skin surface area.[1] In any case, the contribution of the appendages to the overall penetration process still represents a theme of investigation, especially concerning the hair follicles.[2] Studies performed in recent years seem to suggest that follicular drug penetration could be more significant than previously believed.[3–5]

Suitable techniques to determine follicular penetration and storage should allow differentiation between transepidermal and transfollicular penetration.[2] Among the methods used,[3,7–10] a noninvasive method that can also be applied *in vivo* is the cyanoacrylate skin surface biopsy,[11–13] which consists in applying superglue on the skin surface and removing it after polymerization, thus entrapping corneocytes and follicular casts. As this method does not allow distinction between the transepidermal and transfollicular routes, an emerging technique is represented by differential stripping, which consists in subjecting the skin to a tape-stripping technique followed by cyanoacrylate skin surface biopsy.[1,14] The tape-stripping technique is a widely employed method that allows removal of the SC layer by layer, and can be used to determine the amount of a substance retained in the skin surface both *in vitro* and *in vivo*. With the differential stripping technique, it is also possible to quantify SC retention after tape stripping and follicular retention by cyanoacrylate casting, making a complete survey of drug retention in the skin.

Determining follicular retention could be useful in targeted follicular delivery, which includes the treatment of hair growth abnormalities and hair follicle-associated diseases.[15–17]

No exhaustive *in vitro* skin models are currently available to study follicular drug penetration. In fact, human skin, hamster, and pig ear skin, investigated previously,[5,6,18–21] revealed several drawbacks such as, the higher hair follicle intensity per cm^2 in animal species, which influences the follicular penetration rate. Besides, even though pig ear skin seems to represent a more suitable *in vitro* model for the analysis of the follicular storage than excised human skin, porcine hair follicles are larger than human hair follicles and this may lead to an overestimation of the penetration process. Moreover, the excised human skin itself is not devoid of limitations as the excision process leads to the contraction of the reticular fibers, reducing the reticular reservoir.[19,22]

Other studies used rodent skin as a model for targeted follicular delivery.[23–26] Despite many differences that can lead to overestimations of permeability, hairless rat continues to be a valid model for dermatological studies. As the permeability of the scalp in patients with alopecia areata or androgenic

Abbreviations used: FNS, finasteride; HPCH, hydroxypropyl chitosan.

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Table 1. Composition of the Formulations under Study

Batch	P-08–016	P-10–008
Ingredients	% (w/w)	% (w/w)
FNS	0.25	0.25
Ethyl alcohol 96%	55.00	71.25
Propylene glycol	5.00	28.50
Purified water	38.75	–
HPCH	1.00	–

alopecia often reveals a reduced epidermal barrier function, hairless rat skin could be especially suitable as an alternative model for the lesional scalp skin. Although hairless rat skin is slightly different from human skin, the pilosebaceous unit and hair density are comparable[27] and the sebaceous gland density and size are closely related to those of human forehead skin (100–200 per cm^2).[15]

The present investigation aimed at both validating the differential stripping procedure on hairless rat skin as a substrate and at assessing the role of hair follicles in the cutaneous penetration of finasteride (FNS) for the treatment of male baldness after application of one hydroxypropyl chitosan (HPCH)-based formulation (P-08–016) and one anhydrous formulation without HPCH (P-10–008) for either 6 or 24 h. These two formulations previously demonstrated their ability to promote high levels of cutaneous deposition of FNS in the hair bulb region with minimal systemic absorption.[28]

MATERIALS AND METHODS

Chemicals

Two experimental liquid formulations, P-08–016 and P-10–008 (Polichem S.A., Lugano Pazzallo, Switzerland; composition reported in Table 1) were used as received. FNS (batch number J7C002F) was supplied by Polichem S.A. All other chemicals and solvents were of analytical grade.

Animals

Rat skin was obtained from 5-week-old hairless male animals (HsdHanTM:RNU-Foxn1 rnu; Harlan Italy srl, Correzzana, Italy). The animals were killed by cervical dislocation immediately before the experiments, the skin was carefully excised, and the adhering fat and subcutaneous tissue were removed. The study was approved by the Ethics Committee of the University of Pisa and the protocol was compliant with European Union Directive 86/609/EEC for the use of experimental animals.

Tuning and Validation of the Differential Stripping Technique

After positioning the rat skin on a support and delimiting a precise area with a Teflon mask, the skin was subjected to a certain number of tape strips (from 1 to 20). In order to determine how many strips were necessary to completely remove the SC and the viable epidermis, each strip was observed by light microscopy.

After that, a cyanoacrylate biopsy of the stripped skin was performed and the obtained sample was observed microscopically.

Moreover, skin residuals after different numbers of tape strips and after the complete differential stripping procedure were fixed in 10% buffered formalin solution, dehydrated, and embedded in JB-4 plastic resin. Coronal sections were cut by a microtome (Reichert-Jung), mounted onto gelatine-coated slides, and stained with methylene blue/toluidine blue for microscopic examination. The specimens were assessed under a Leitz Diaplan microscope.

In Vitro Deposition Experiments

In vitro permeation tests through excised rat skin were carried out as previously described[29] using Gummer-type diffusion cells with an available diffusion area of 1.23 cm^2 and the SC facing the donor compartment. Two hundred microliters of each formulation, whose composition is reported in Table 1, was placed on the skin surface. The receiving phase (5.0 mL) was isotonic phosphate buffer saline (66.7 mM, pH 7.4) containing 0.003% (w/v) sodium azide to prevent bacterial growth, maintained at 37°C and stirred at 600 rpm. At predetermined time intervals, the receiving phase was completely changed and replaced with fresh fluid to maintain sink conditions. The experiments lasted 6 or 24 h and were replicated five times.

At the end of the *in vitro* permeation experiments, the skin was removed from the diffusion cells, rinsed with distilled water to remove excess formulation from the skin surface and gently wiped with cotton-wool tampons. The skin specimens were then positioned on a homemade specific apparatus delimiting the drug-exposed surface. Afterward, the skin was subjected first to tape stripping and then to cyanoacrylate skin surface biopsy.

Adhesive Tape-Stripping Technique

The tape-stripping procedure was performed as described by Weigmann et al.[30] The skin was stripped using an adhesive tape (Tesa film N. 5529; Beiersdorf, Hamburg, Germany) and the tape strips were pressed on the skin by applying uniform pressure in order to obtain intimate contact between the film and the skin. The first tape strip was discarded, as this represents unabsorbed material only. Then, the subsequent strips were carefully removed and the entire procedure was repeated 15 times (tape strips no. 1–15).

Cyanoacrylate Skin Surface Biopsy

Following the removal of 15 tape strips, a drop of Superglue (UHU GmbH & Co. KG, Bühl/Baden, Germany) was placed on the skin stripped area and the glue was covered with the adhesive tape under slight pressure. After 10 min, the cyanoacrylate polymerized and the strip was removed with a single quick motion, entrapping the casts of the hair follicles.

Each tape-strip sample removed from the treated skin area and each sample derived from cyanoacrylate skin surface biopsy was cut to a size of 1.9 × 3.0 cm^2 and placed in a glass vial containing 5 mL ethanol, sonicated for 10 min, and submitted to centrifugation (15 min, 4,000 rpm). The supernatant was collected for HPLC analysis.

Analytical Methods

The quantitative determination of FNS recovered in skin samples was carried out by HPLC. The apparatus consisted of a Shimadzu (Kyoto, Japan) LC-20AD system with an UV SPD-10A detector equipped of autosampler SIL-10AD VP and a computer integrating system. The injection valve was a Rheodyne with a capacity of 20 μL. A Luna® C8 (10 μm; 250 × 4.6 mm) column was employed. The mobile phase consisted of a mixture of acetonitrile:methanol:water (20:40:40). The detection

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