DOI: 10.1111/exd.12113
www.wileyonlinelibrary.com/journal/EXD

Approach towards developing a novel procedure to selectively quantify topically applied substances in the hair follicles of the model tissue porcine ear skin

Fanny Knorr, Alexa Patzelt, Heike Richter, Sabine Schanzer, Wolfram Sterry and Juergen Lademann

Department of Dermatology, Venerology and Allergology, Center of Experimental and Applied Cutaneous Physiology (CCP), Charité – Universitätsmedizin Berlin, Berlin, Germany
Correspondence: Prof. Dr. Dr.-Ing. Juergen Lademann, PhD, Center of Experimental and Applied Cutaneous Physiology (CCP), Department of Dermatology, Venerology and Allergology, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany, Tel.: +49 30 450518100, Fax: +49 30 450518918, e-mail: juergen.lademann@charite.de

Abstract: Hair follicles represent reservoirs for localized drug therapy and transport pathways for systemic drug delivery. This study describes an approach towards developing a novel procedure for quantifying topically applied substances located in the hair follicles of porcine ear skin, a model for human *in vivo* skin, using a fluorescent dye. Approximately 5% of the topically applied dye was recovered from the hair follicles, which is in accordance with a previous study.

Key words: drug delivery – fluorescence – hair follicles – porcine ear skin – rhodamine B isothiocyanate

Accepted for publication 7 February 2013

Background

It is well established that topically applied substances penetrate into the skin by diffusing into the lipid matrix of the stratum corneum and spreading into the upper corneocyte layers (1,2). Continuous sloughing off of corneocytes through desquamation results in the rapid depletion of topically applied drugs located in the horny layer. Hair follicles play an important role in the penetration of substances into the skin by acting as shunt routes to deeper skin layers (3–5) and as a reservoir for dermally applied substances (6). Interest in the hair follicles as target structures is aimed primarily at their use as drug depots for localized therapy, particularly for the treatment of skin diseases, follicle-related disorders and hair growth abnormalities (7–11). Considerable effort has also focused on exploiting these structures for systemic drug delivery. Drugs administered to the skin that reach the dense network of capillaries surrounding the hair follicles (12) may permeate into the central circulatory system, resulting in the dispersion of the drugs throughout the body (13).

Questions addressed

The quantification of therapeutic agents located in the follicular canals is crucial for questions pertaining to administration, concentrations and dosages. The established method of differential stripping (14) is used to selectively and differentially quantify drugs dispersed throughout the upper layers of the stratum corneum and within appendageal structures. Differential stripping does not provide valid results for *in vitro* studies using the model tissue porcine ear skin, as it is not possible to remove complete follicular casts from porcine skin without producing tears in the cyanoacrylate. For follicular penetration experiments, excised porcine ear skin is a particularly suitable alternative to human skin for the testing of substances with unknown or potentially harmful effects, as its follicular reservoir capacity corresponds to that of living human skin. The excision of human skin from its donor leads to the contraction of elastic fibres surrounding the hair follicles, diminishing the *in vitro* follicular reservoir to 9.5% of the *in vivo* capacity (15). In contrast, the follicular reservoir of porcine ear skin remains constant after the removal of the ears from cadavers as it is fixed on an underlying cartilage. The approach of this study was therefore to aim towards developing a procedure that enables the selective quantification of drugs located in the follicular canals of porcine skin subsequent to topical application.

Experimental design

Marked skin regions of 10 porcine ears were treated with defined amounts of hydroxyethylcellulose gel containing 0.1% rhodamine B isothiocyanate (RhBITC), a derivative of the fluorone dye rhodamine and a labelling agent soluble in ethanol. An exposure time of 30 min allowed for the penetration of the gel into the skin samples subsequent to standardized massage application for 3 min. Tape stripping (16) was performed on each specimen to remove the stratum corneum along with any dye dispersed throughout the upper cell layers. The successive removal of the dye from the skin surface by tape stripping was verified by examining the 1st, 10th, 25th, 40th and 80th strips using fluorescence microscopy. After tape stripping, biopsies were punched from the stripped regions of each ear, detached from the underlying cartilage and submerged in ethanol for 24 h. Additional biopsies were taken from untreated skin regions as controls. All specimens were subjected to ultrasound for 60 min and were centrifuged at 4000 rpm for 10 min, after which the biopsies were resubmerged in fresh ethanol and the extraction process was repeated. Fluorescence found in the supernatants was detected using fluorescence spectroscopy. Measurements yielded relative concentrations of follicular RhBITC, which were calculated into absolute values using a calibration curve. Subsequent to extraction, the biopsies were examined under the fluorescence microscope for remaining dye.

Results

The amounts of corneocytes and fluorescence visualized on the strips using the transmission and fluorescence modus, respectively, decreased substantially as stripping progressed. It can be assumed that the isolated spots of fluorescence found on the 80th strips

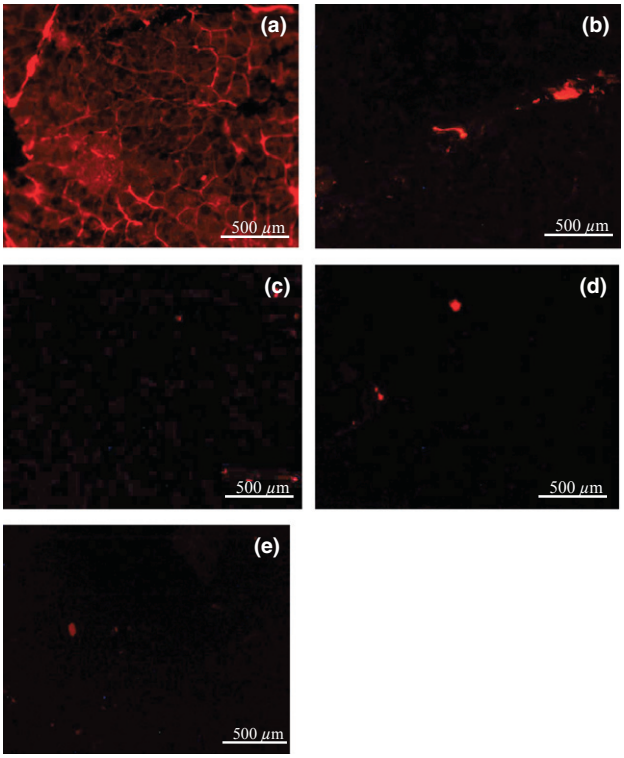

Figure 1. Detection of RhBITC in the stratum corneum and follicular orifices on the 1st (a), 10th (b), 20th (c), 60th (d) and 80th (e) tape strip.

corresponded to RhBITC located in the follicular orifices (Fig. 1), as corneocytes were no longer detectable using the transmission mode. The results pertaining to the experimental controls indicated the presence of only trace amounts of fluorescence, which can be ascribed to skin fluorophores. Fluorescence emitted by the experimental extraction solutions can thereby be attributed to follicular RhBITC. Repeated extraction yielded no further fluorescence. On average, 5.01 (±1.86)% of the dye administered to the skin was detected in the follicular extraction solutions. This is in accordance with a previous study, in which 5% of topically applied sodium fluorescein was extracted from the follicular casts of human subjects by differential stripping (14). Despite its solubility in ethanol and although no fluorescence could be detected on the biopsies after extraction using the fluorescence microscope, the ability of RhBITC to covalently bind to proteins must be considered, as it is conceivable that small amounts of the dye remained bound within the hair follicles after extraction.

Conclusion

The present procedure describes an approach towards the development of a novel procedure for the selective quantification of substances located in hair follicles of porcine ear skin. Additional research using different model substances is necessary.

Acknowledgements

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

Author contributions

This study was designed by A. Patzelt and W. Sterry. The research was performed by F. Knorr, S. Schanzer, H. Richter and J. Lademann. F. Knorr, A. Patzelt and J. Lademann analysed the data. The article was written by F. Knorr, A. Patzelt and J. Lademann.

Conflict of interests

The authors report no conflict of interest relevant to the subject of this study.

References

1 Bouwstra J, Pilgram G, Gooris G *et al.* Skin Pharmacol Appl Skin Physiol 2001: **14** (Suppl 1): 52–62.
2 Hadgraft J. Int J Pharm 2001: **224**: 1–18.
3 Dokka S, Cooper S R, Kelly S *et al.* J Invest Dermatol 2005: **124**: 971–975.
4 Jung S, Otberg N, Thiede G *et al.* J Invest Dermatol 2006: **126**: 1728–1732.
5 Otberg N, Teichmann A, Rasuljev U *et al.* Skin Pharmacol Physiol 2007: **20**: 195–198.
6 Lademann J, Knorr F, Richter H *et al.* Skin Pharmacol Physiol 2008: **21**: 150–155.
7 Munster U, Nakamura C, Haberland A *et al.* Pharmazie 2005: **60**: 8–12.
8 Domashenko A, Gupta S, Cosartelis G. Nat Biotechnol 2000: **18**: 420–423.
9 Hoffman R M. Nat Biotechnol 2000: **18**: 20–21.
10 Lieb M L, Limatta A P, Bryan R N *et al.* J Pharm Sci 1997: **86**: 1022–1029.
11 Li L, Hoffman R M. Nat Med 1995: **1**: 705–706.
12 Ryan T: Cutaneous circulation. In: Goldsmith L A, ed. Biochemistry and Physiology of the Skin. London: Oxford University Press, 1983: 817–877.
13 Lauer A C, Ramachandran C, Lieb L M *et al.* Adv Drug Deliv Rev 1996: **18**: 311–324.
14 Teichmann A, Jacobi U, Ossadnik M *et al.* J Invest Dermatol 2005: **125**: 264–269.
15 Patzelt A, Richter H, Buettemeyer R *et al.* Eur J Pharm Biopharm 2008: **70**: 234–238.
16 Weigmann H J, Lademann J, Meffert H *et al.* Skin Pharmacol Appl Skin Physiol 1999: **12**: 34–45.