

Topical Bioavailability of Triamcinolone Acetonide: Effect of Occlusion

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Key Words

Occlusion · Topical bioavailability · Reservoir · Tape stripping · Triamcinolone acetonide

Abstract

Background/Aims: Occlusion by covering the skin with an impermeable wrap enhances skin hydration, affects drug absorption and can induce the formation of a drug reservoir within the stratum corneum. This is desired in local therapy with topical corticosteroids. The aim of the study was to investigate the effect of occlusion before (experiment 1) and after (experiment 2) application on the penetration of triamcinolone acetonide (TACA) into the stratum corneum. **Methods:** The experiments were conducted on the forearms of 10 healthy volunteers. In experiment 1, 100 µg/cm² TACA in acetone were applied on 3 sites per arm, one arm having been pre-occluded for 16 h. In experiment 2, the same dose was applied on 2 sites per arm, and one arm was occluded after application until skin sampling. Stratum corneum samples were removed by tape stripping at 0.5, 4 and 24 h (experiment 1) and 4 and 24 h (experiment 2) after application. Corneocytes and TACA were quantified by ultraviolet-visible spectroscopy and HPLC, respectively. The total TACA amount penetrated into the stratum corneum was evaluated by multifactor ANOVA. **Results:** TACA penetration into the stratum corneum with and without pre-occlusion (experiment 1) showed no significant difference and decreased with time.

Occlusion after application (experiment 2) produced a marked TACA accumulation within the stratum corneum, which persisted for 24 h. **Conclusion:** Pre-occlusion showed no effect on the topical bioavailability of TACA in the stratum corneum. In contrast, post-occlusion enhanced the TACA penetration by a factor of 2, favouring the development of a drug reservoir.

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Introduction

Occlusion by covering the skin with an impermeable wrap inhibits the physiological water loss through the skin and increases the skin temperature [1]. The enhanced skin hydration can induce morphological changes of the stratum corneum such as swelling of the corneocytes [2], water uptake into intercellular lipid domains [3] and deepening of skin furrows [4].

Such changes considerably affect the percutaneous absorption of topically applied drugs. Vickers [5] observed in the early 60s that the application of corticosteroids under occlusion promoted the formation of a long-lasting drug reservoir within the stratum corneum. This is a welcome effect in local therapy with topical corticosteroids. The longer the active drug is present within the skin, the longer a therapeutic effect can be expected.

Occlusion does not enhance the percutaneous absorption of all drugs [6, 7]. The enhancement appears to be compound and vehicle specific [8]. Volatile solvents such as acetone and ethanol seem to induce a clear penetration enhancement into the stratum corneum after application under occlusion [9].

The aim of the study was to investigate the effect of occlusion on the *in vivo* penetration of triamcinolone acetonide (TACA), a moderately potent corticosteroid, into the stratum corneum, using standardized tape stripping as sampling technique and validated analytical methods – ultraviolet-visible (UV/VIS) spectroscopy, HPLC – for the quantification of both corneocytes and TACA. In experiment 1, the effect of occlusion prior to topical application of TACA in acetone (pre-occlusion) was investigated. In experiment 2, the effect of occlusion after TACA application (post-occlusion) was investigated.

Subjects and Methods

Material and Formulation

A solution of 4.2 mg/ml micronized TACA Ph.Eur. (Caesar & Loretz GmbH, Hilden, Germany) in acetone Ph.Eur. (Hänseler AG, Herisau, Switzerland) was prepared according to current GMP guidelines [10].

Subjects and Study Design

A total of 10 healthy adult volunteers with skin phototype II–III (Caucasian) without excessive hairiness of the inner forearm were recruited and underwent a dermatological examination 1 week prior to study start. The experiments were conducted on the volar aspect of the forearms during 2 days as an open, explorative study with half-side intra-individual comparison. In experiment 1, one arm was pre-occluded prior to TACA application by wrapping it for 16 h in polypropylene film (Tangan® wrap). In experiment 2, occlusion of one arm followed TACA application, and the occlusive wrap was not removed until tape stripping. In both experiments, the contralateral arm served as control and was not occluded. Finite single doses (100 µg/cm²) of TACA solution were applied on selected skin areas, which were afterwards sampled by tape stripping at different times following the protocol described below. Within 1 month after tape stripping, the wound healing was evaluated in a final dermatological examination. Figure 1 displays the flow chart of the study.

The study was conducted according to the ethical rules stated in the Declaration of Helsinki and was approved by the local ethical committee and the national authorities (Swissmedic). The volunteers signed written consents for participation.

Application of the Formulation

In experiment 1, three skin sites per arm were treated (total of 6 skin sites per volunteer). In experiment 2, two skin sites per arm were treated (total of four skin sites per volunteer). The application area was delineated by a rectangular glass frame (10.5 cm²)

glued onto the skin (Sauer® skin glue; Manfred Sauer GmbH, Lobbach, Germany). A volume of 250 µl formulation, corresponding to a TACA dose of 100 µg/cm², was uniformly applied to the delineated area with a Hamilton syringe (Supelco, Buchs, Switzerland). The vehicle was allowed to evaporate. Skin sites not stripped within 0.5 h after application were protected with non-occlusive cotton gauzes until tape stripping. To avoid drug dislocations within skin, no skin washing was performed to remove excess of formulation, and the volunteers were not allowed to shower during the 2-day experiment.

Skin Sampling by Tape Stripping

Stratum corneum samples were removed by standardized tape stripping [11] with Tesa tape (Tesa Multi-Film Crystal-Clear® 57315, 19 mm width, Beiersdorf, Hamburg, Germany) at 0.5, 4 and 24 h (experiment 1) and at 4 and 24 h (experiment 2) after application (fig. 1). A template delineating an area of 5.6 cm² was fixed onto the skin. Tapes were successively placed onto this area and stripped off with a rapid movement until the entire stratum corneum had been removed. A hand roller supplying a constant pressure of 140 g/cm² was passed 10 times over each tape before removal. Total stratum corneum removal was defined as light transmission through the tape ≥95%, measured by UV/VIS spectroscopy as described below.

Analytics

The amount of corneocytes adhering to each tape was quantified directly on the tapes at 430 nm using a spectrophotometer (Lambda 35; Perkin Elmer, Überlingen, Germany), custom-modified to obtain a rectangular light beam of 1 cm², as described in Weigmann et al. [12]. The quantification of the corneocytes is required for the calculation of penetration profiles.

The amount of TACA adhering to each tape was quantified by an ICH-validated HPLC method [13] after extraction with 1.5 ml 60% methanol on a horizontal shaker (30 min, 140 rpm, Heidolph Unimax 2010; Heidolph, Germany). The protective gauzes were similarly extracted with 10 ml 60% methanol. A Symmetry Shield™ RP18 column and a Waters Alliance HPLC system (2690 Separation Module, 996 Photodiode Array Detector), all Waters Corporation, Millford, Mass., USA, were used. Methanol 60% (vol/vol) with a flow rate of 0.3 ml/min was the mobile phase, and the injection volume of the samples was 20 µl. Quantification of TACA occurred at 240 nm. The limit of quantification (LOQ) was 100 ng/ml (corresponding to 27 ng/cm²), the limit of detection 35 ng/ml (9 ng/cm²). Both repeatability and intermediate precision of the method were ≤2% in the working range without LOQ (0.5–20 µg/ml) and ≤5% in the whole range including LOQ (0.1–20 µg/ml). The overall accuracy was 93 ± 5%.

Data Analysis

Sample Size

The method deviation (intra-individual standard deviation) had been determined previously and was ±40%. To provide a power of 50% in detecting a 50% difference between the 2 treatment groups at the 5% significance level, a total of 5 volunteers were needed according to the two-sided t test nomogram for paired values after logarithmic transformation [14] and were thus enrolled in each experiment.

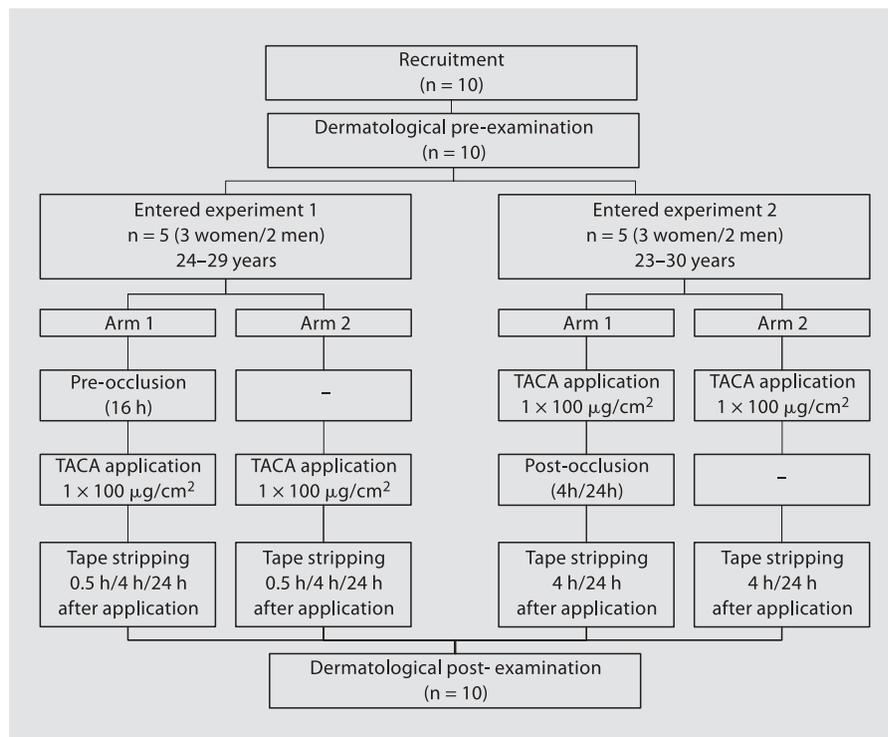


Fig. 1. Flow chart of the study: from recruitment to dismissal of the volunteers. The effect of pre-occlusion (experiment 1) and post-occlusion (experiment 2) on the TACA penetration into the stratum corneum was investigated.

Quantitative TACA Penetration into the Stratum Corneum

The TACA amounts on each tape (area 5.6 cm²) of each skin site were added up to the total TACA amount penetrated into the stratum corneum, which was evaluated statistically. The significance of differences between the treatment groups at each time was tested after logarithmic transformation in a multifactor variance analysis (ANOVA) [15] with the following factors: volunteer, time, application and the interaction time/application. For the factors displaying a statistical significance in ANOVA ($p < 0.05$), a post hoc comparison was performed with the least significance difference test. Two different types of evaluation were performed: the evaluation of (a) the total TACA amount within the stratum corneum (sum of TACA amounts on all tapes) and of (b) the TACA amount without tapes 1–3 (on which formulation excess, e.g. TACA crystals, could be located). Statgraphics® Plus 5 software (Manugistic Inc., Rockville, Md., USA) was used to conduct the analysis of the trial.

Qualitative TACA Penetration into the Stratum Corneum: Penetration Profiles and Photographic Recording

To visualize the drug distribution within the stratum corneum, TACA amounts quantified on each tape were correlated with the tape number and with the depth of penetration into the stratum corneum. Removal of the entire stratum corneum is a prerequisite for the profile calculation, since the sum of corneocyte (pseudo-)absorbance on one skin site represents 100% stratum corneum. Thus, the relative amount of stratum corneum removed by each tape can be calculated from the individual absorbance values as fully described in Jacobi et al. [16] and Weigmann et al. [17].

The stratum corneum removal pattern was photographically documented. All photographs were taken at a standard camera-object distance.

Results

Demographics of the Subjects

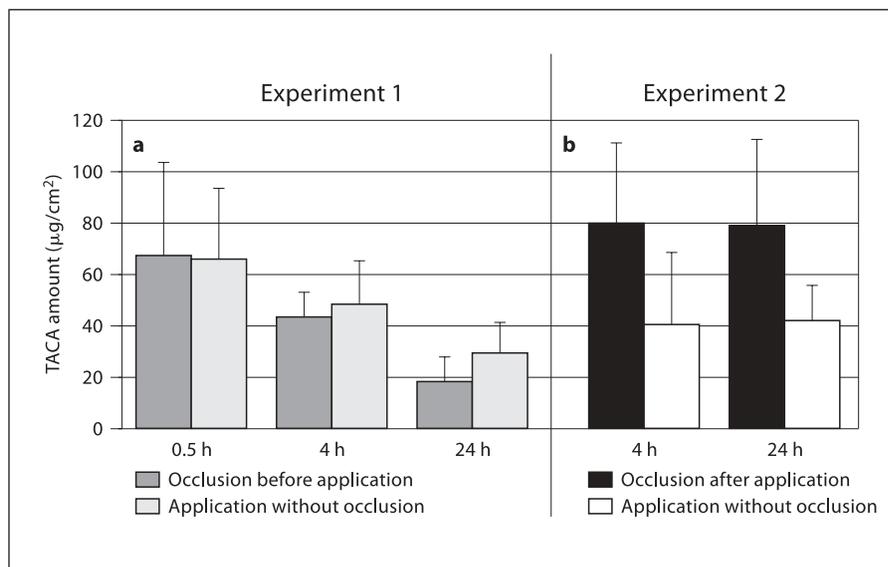
A total of 10 healthy adult volunteers (5 male and 5 female) aged 23–30 years (mean 25) were recruited and finished the study. Five volunteers were assigned to each experiment (fig. 1). The tape stripping experiments were conducted from April 2004 to July 2004 in Basel. The stripped skin sites showed a good wound healing and no scarring at the final dermatological investigation. Slight transient hyperpigmentation was observed in some volunteers.

Quantitative TACA Penetration into the Stratum Corneum

Experiment 1: Effect of Pre-Occlusion

Pre-occlusion showed no effect on the TACA penetration into the stratum corneum (fig. 2a). The penetrated TACA amount decreased significantly with time after both application modes, with and without pre-occlusion

Fig. 2. Total TACA amount penetrated into the stratum corneum after application of $100 \mu\text{g}/\text{cm}^2$ TACA in acetone under non-occluded versus pre-occluded conditions (a, experiment 1) and under non-occluded versus post-occluded conditions (b). Mean and standard deviation are displayed.



($p < 0.001$). At 0.5 h, mean TACA amounts of 66 ± 28 and $67 \pm 37 \mu\text{g}/\text{cm}^2$ were quantified within the stratum corneum of non-occluded and pre-occluded skin, respectively. At 4 h, mean values of 48 ± 17 and $43 \pm 10 \mu\text{g}/\text{cm}^2$ were recovered in non-occluded and pre-occluded skin, and at 24 h values of 29 ± 12 and $18 \pm 10 \mu\text{g}/\text{cm}^2$.

By excluding tapes 1–3, the TACA amounts were approximately halved: at 0.5 h, mean values of 30 ± 12 and $35 \pm 16 \mu\text{g}/\text{cm}^2$ were quantified in non-occluded and pre-occluded skin, respectively, at 4 h values of 25 ± 8 and $24 \pm 8 \mu\text{g}/\text{cm}^2$ and at 24 h values of 20 ± 9 and $12 \pm 7 \mu\text{g}/\text{cm}^2$.

Independently of the application mode (with/without pre-occlusion), 8–13% of the applied TACA amount was recovered in the gauzes used to protect the treated skin sites.

Statistical evaluation by ANOVA displayed a significant difference only for the factor time ($p < 0.001$), whereas the factors volunteer, application and the interaction time/application showed no influence (for both evaluations: all tapes and without tapes 1–3).

Experiment 2: Effect of Post-Occlusion

Post-occlusion induced a marked accumulation of TACA within the stratum corneum. The amount of TACA recovered in the stratum corneum after post-occlusion showed a highly significant difference ($p < 0.01$) compared to non-occluded skin (fig. 2b).

Over time, the TACA amount within the stratum corneum remained constant after both application modes:

after normal application (without occlusion), the values amounted to 40 ± 28 and $42 \pm 14 \mu\text{g}/\text{cm}^2$ at 4 and 24 h, respectively. After post-occlusion, a twofold higher TACA amount was quantified within the stratum corneum, with values amounting to 80 ± 32 and $79 \pm 33 \mu\text{g}/\text{cm}^2$ at 4 and 24 h, respectively.

A similar trend was also seen after discarding tapes 1–3: after normal application, TACA amounts of 21 ± 12 and $24 \pm 11 \mu\text{g}/\text{cm}^2$ were quantified at 4 and 24 h, versus TACA amounts of 37 ± 13 (at 4 h) and $42 \pm 28 \mu\text{g}/\text{cm}^2$ (at 24 h) after application followed by post-occlusion.

The TACA amount extracted from the gauzes displayed values of 11% of the applied dose after normal application and 3–7% after application under occlusion.

Qualitative TACA Penetration into the Stratum Corneum: Penetration Profiles and Photographic Recording

Two exemplary penetration profiles of TACA into the stratum corneum at 24 h after normal and post-occluded application are depicted in fig. 3. The profiles show that TACA permeated the stratum corneum and reached deeper tissues. The higher TACA amount retained within the stratum corneum after application under post-occlusion is clearly visible.

The increased hydration of the skin due to occlusion caused a decreased corneocyte cohesion. Fewer tapes were required to remove the entire stratum corneum of occluded skin sites, since larger amounts of corneocytes were removed on single tapes. This happened in both ex-

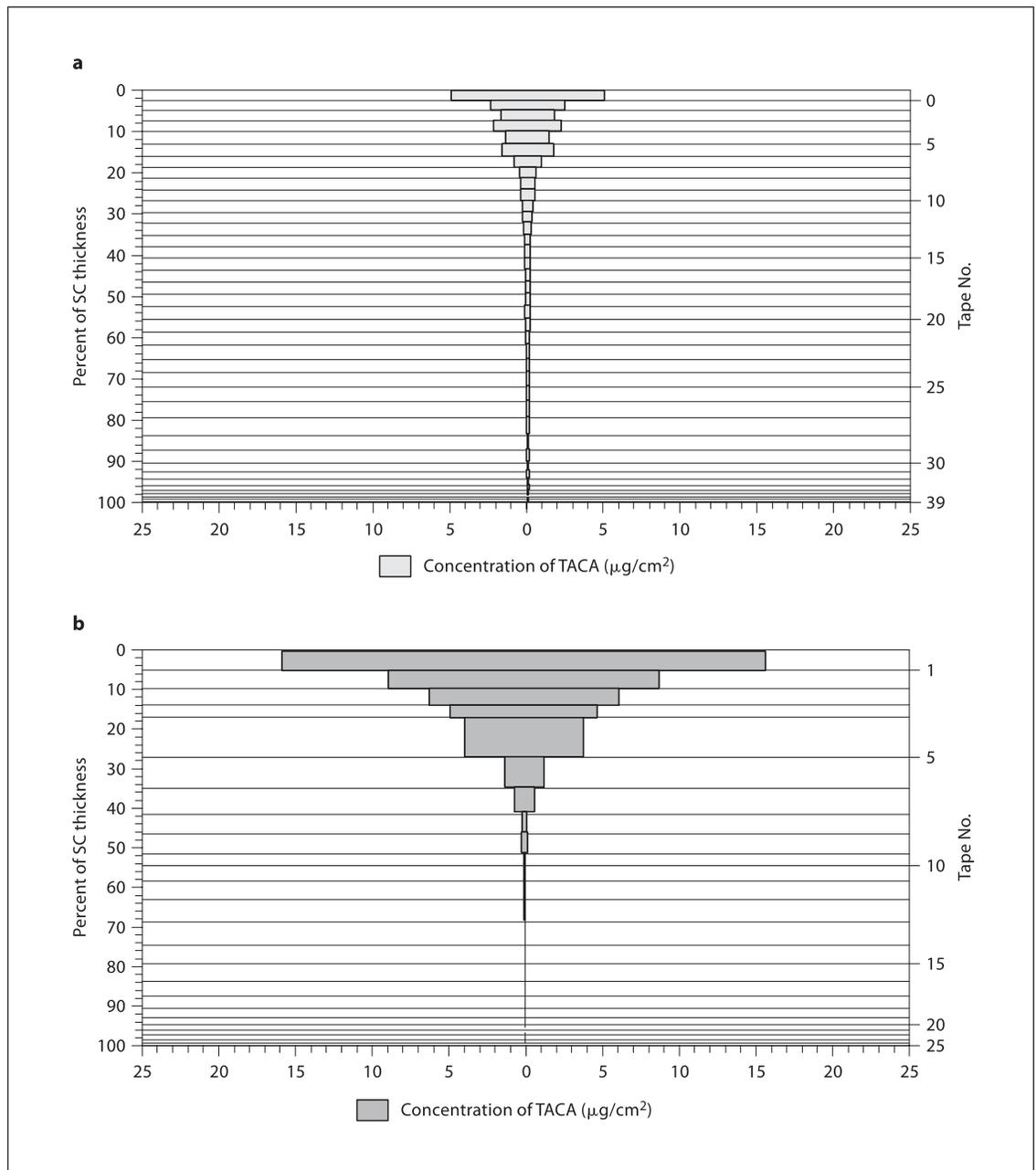


Fig. 3. Typical penetration profiles of TACA into the stratum corneum (SC) 24 h after normal application (a) and after application followed by occlusion in the same volunteer (b). TACA amounts on each tape (horizontal light or dark grey bars) are correlated with the tape number (right scale) and with the depth of penetration into the stratum corneum, displayed as percentage of the total stratum corneum thickness (left scale).

periments 1 and 2 after long-time occlusion and tape stripping following within 0.5 h the removal of the occlusive wrap. The removal of larger amounts of corneocytes mostly occurred between tapes No. 5 and 10 (fig. 4).

Discussion

In the present experiments, the topical bioavailability of TACA was described by the TACA penetration into the stratum corneum during time. Because the stratum cor-

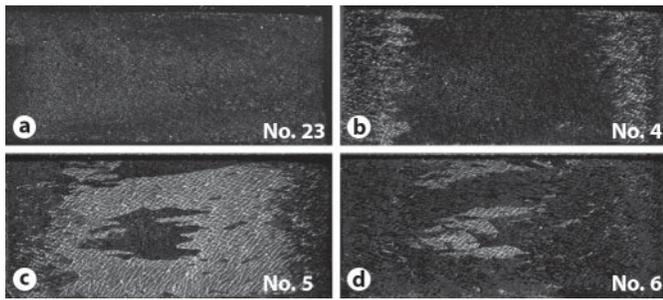


Fig. 4. Photographs of stripped tapes. Long-time occlusion induced a loosening of the stratum corneum structure. Larger amounts of corneocytes (skin sheets) were removed on single tapes. **a** 23rd tape stripped from a non-occluded skin site: a 'normal', homogeneous amount of skin is removed. **b–d** 4th–6th tapes stripped from a skin site occluded for 24 h: larger amounts of corneocytes are removed as sheets on single tapes. This artefact due to occlusion does not disturb the assessment of topical bioavailability, provided that the entire stratum corneum of one skin site is sampled.

neum is the rate-limiting barrier of the skin, the TACA amount within the stratum corneum may be correlated with the drug amount at the target site (viable epidermis, dermis). Two different modes of occlusion were investigated: pre-occlusion (occlusion before TACA application) and post-occlusion (occlusion after TACA application). Due to the good solubility of TACA in acetone and to its frequent use as a model vehicle in skin research [18–20], acetone was chosen as solvent. Moreover, volatile vehicles such as acetone allow the application of a finite, solvent-deposited drug amount [21, 22] and seem to induce a penetration enhancement after application under occlusion [9].

Experiment 1 (pre-occlusion) investigated the application on occluded and thus more hydrated skin, as it could be the case after an extensive bath. Pre-occlusion did not show any effect on the TACA penetration into the stratum corneum compared to normal application. Agner and Serup [23] observed that the effect of occlusion on the stratum corneum hydration is transitory, transepidermal water loss returning to baseline values within 0.5–1 h after removal of the occlusive device. Accordingly, in our experiment 1, larger amounts of corneocytes (skin sheets) due to the increased skin hydration and to the disruption of the stratum corneum were only observed at the first stripping time (0.5 h), when the occlusive wrap had just been removed. At later times (4 and 24 h), the corneocyte amount removed by the tapes was homogeneous.

The disruptive effect of water on the stratum corneum structure was especially observed after the stripping of about 5–10 tapes, whereas the outer- and the innermost stratum corneum seemed to be less affected. Accordingly, swelling of corneocytes and formation of water pools between the cells in the middle stratum corneum could be observed microscopically by Bouwstra et al. [3] after 24-hour hydration of the stratum corneum isolated from dermatomed skin.

Experiment 2 (post-occlusion) investigated the application followed by occlusion, which can be clinically useful to improve the effect of topical corticosteroids in severe forms of skin diseases. A 'physiological' occlusion is possible as well in intertriginous skin areas, and also certain vehicles (e.g. ointments) can be occlusive. Post-occlusion enhanced the TACA retention within the stratum corneum by a factor of 2, favouring the development of a drug reservoir. The TACA amount retained by the stratum corneum remained constant between 4 and 24 h, and still amounted to 80% of the applied dose 24 h after application. The amount of drug quantified in the gauze used to protect the treated sites until tape stripping was slightly lower after occlusion, showing that occlusion may have promoted the dissolution of drug crystals located on the skin surface.

The post-occlusion results obtained in our investigations agree with preliminary observations of Carr and Wieland [24]. They investigated the percutaneous penetration of ^{14}C -labelled TACA applied in 95% ethanol on a single male volunteer. After 1 day, 81% of the applied dose was found within the stratum corneum after occlusion versus 38% without occlusion. Our trial performed with a larger number of volunteers and a different volatile vehicle yielded similar results. The extent of the topical bioavailability of TACA was significantly enhanced by post-occlusion, and the release rate from the stratum corneum into deeper skin layers was delayed because of the temporary accumulation of TACA within the hydrated stratum corneum (reservoir formation).

The formation, the extent and the maintenance of a reservoir are highly supported by application of topical compounds under occlusion [5]. The enhanced skin hydration due to occlusion can considerably change the affinity of the applied compound to the stratum corneum compartment and its partitioning between the different skin compartments [25]. Moreover, morphological changes (e.g. swelling of the corneocytes, water pool formation) due to overhydration seem to enhance the maintenance of the stratum corneum reservoir. After removal of the occlusive wrap, the hydration level of the stratum

corneum rapidly returns to normal levels, and the facilitated partitioning between stratum corneum (lipophilic by nature but hydrated by occlusion) and epidermis (hydrophilic by nature) is normalized as well. Thus, topically applied compounds may temporarily remain trapped within the stratum corneum. Our results show that occlusion must follow drug application (post-occlusion) to induce a reservoir, whereas occlusion preceding drug application (pre-occlusion) is not sufficient.

The standardized tape stripping technique in combination with the quantification of corneocytes by UV/VIS spectroscopy and the quantification of TACA by HPLC has been shown to be adequate for the quantification of TACA within the stratum corneum. This technique is not influenced by artefacts due to occlusion and does not require radiolabelling of the drug.

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Conclusions

Pre-occlusion showed no effect on the topical bioavailability of TACA in the stratum corneum. In contrast, occlusion after application enhanced the TACA penetration into the stratum corneum by a factor of 2, favouring the development of a drug reservoir.

Acknowledgements

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