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ORIGINAL PAPER

Topical bioavailability of triamcinolone acetonide: effect of dose and application frequency

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Abstract The application frequency of topical corticosteroids is a recurrently debated topic. Multiple-daily applications are common, although a superior efficacy compared to once-daily application is not unequivocally proven. Only few pharmacokinetic studies investigating application frequency exist. The aim of the study was to investigate the effect of dose (Experiment 1) and application frequency (Experiment 2) on the penetration of triamcinolone acetonide (TACA) into human stratum corneum (SC) in vivo. The experiments were conducted on the forearms of 15 healthy volunteers. In Experiment 1, single TACA doses (300 μg/cm^2 and 100 μg/cm^2) dissolved in acetone were applied on three sites per arm. In experiment 2, single (1×300 μg/cm^2) and multiple (3×100 μg/cm^2) TACA doses were similarly applied. SC samples were harvested by tape stripping after 0.5, 4 and 24 h (Experiment 1) and after 4, 8 and 24 h (Experiment 2). Corneocytes and TACA were quantified by UV/VIS spectroscopy and HPLC, respectively. TACA amounts penetrated into SC were statistically evaluated by a paired-sample *t*-test. In Experiment 1, TACA amounts within SC after application of 1×300 μg/cm^2 compared to 1×100 μg/cm^2 were only significantly different directly after application and similar at 4 and 24 h. In Experiment 2, multiple applications of 3×100 μg/cm^2 yielded higher TACA amounts compared to a single application of 1×300 μg/cm^2 at 4 and 8 h. At 24 h, no difference was observed. In conclusion, using this simple vehicle, considerable TACA amounts were retained within SC independently of dose and application frequency. A low TACA dose applied once should be preferred to a high dose, which may promote higher systemic exposure.

Keywords Application frequency · Reservoir · Triamcinolone acetonide · Tape stripping · Topical bioavailability

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Introduction

In current dermato-pharmacotherapy, the application frequency of topical corticosteroids is a recurrently debated topic. Several studies have been performed to propose recommendations for optimal therapy. Once- or twice-daily applications are common [6], and many dermatologists usually follow twice-daily applications, although a superior efficacy of a multiple-daily application is not unequivocally proven. Recently, a systematic review reported a similar efficacy of once-daily versus multiple-daily applications of topical corticosteroids of the same potency in atopic dermatitis [7]. In addition to pharmacodynamic investigations, only few pharmacokinetic studies investigating application frequency exist (reviewed in [28]). Unfortunately, these studies make their statements on data derived from drug concentration determination in plasma [1, 20] and urine [11, 27]. This information is incomplete because

these data document systemic bioavailability and not topical bioavailability.

Topical bioavailability can be estimated from the drug concentration within the stratum corneum, which is expected to be related to the drug concentration at the target site (i.e. usually viable epidermis or dermis) since the stratum corneum is the rate limiting barrier for percutaneous absorption. Similarly to the determination of the drug concentration in blood and/or urine as surrogate for the concentration at the target tissue, the determination of the drug concentration in the stratum corneum may serve as a surrogate for the concentration in the viable (epi-)dermis [17]. Tape stripping, which enables the removal of the stratum corneum layer by layer, is a useful dermato-pharmacokinetic technique for the assessment of drug amounts in stratum corneum as a function of time [19].

Human stratum corneum has the property to store previously applied drugs depending on the drug, the formulation, the application procedure, and the state of the skin [15]. Drug accumulation in the skin forms a reservoir, from which minor amounts are released during a prolonged time period. The existence of a reservoir within the stratum corneum has been documented for several xenobiotics [15], particularly for topical corticosteroids [22]. This is a welcome phenomenon for topical corticosteroids and affects the choice of application frequency and dose.

Our investigation is focused on topical bioavailability. We determined the in vivo penetration of triamcinolone acetonide (TACA), a moderately potent corticosteroid, into human stratum corneum after different application modes in a simple vehicle (acetone). The investigation was divided into two parts. In Experiment 1, the influence of the dose was investigated by comparing the TACA penetration into stratum corneum after application of a high (1×300 μg/cm^2) and a low (1×100 μg/cm^2) TACA dose. In Experiment 2, the influence of application frequency was investigated by comparing the TACA penetration after once-daily application of a high TACA dose (1×300 μg/cm^2) to the TACA penetration after thrice-daily application of a low TACA dose (3×100 μg/cm^2).

Subjects and methods

Material and formulations

Micronized TACA Ph.Eur. was purchased from Caesar and Loretz GmbH, Hilden, Germany and acetone Ph.Eur. from Hänseler AG, Herisau, Switzerland. Solutions of 4.2 mg/ml (for the application of 100 μg/cm^2) and 12.6 mg/ml (application of 300 μg/cm^2) TACA in acetone were prepared following current GMP guidelines.

Subjects and study design

A total of 15 healthy adult volunteers with skin phototype II-III (Caucasian) and with minor hairiness of the volar aspect of the forearm were recruited and underwent a preliminary dermatological examination one week prior to study start. The experiments were conducted on the volar aspect of the forearms during 2 days as an open study with half-side intra-individual comparison. Experiment 1 (influence of dose) was regarded as explorative, whereas Experiment 2 (influence of application frequency) was the main investigation. TACA in acetone was applied on selected skin sites, from which the stratum corneum was harvested afterwards by tape stripping at 3 different time points according to the protocol described below. No skin treatments were allowed during 24 h before study start, and volunteers were not allowed to shower or practise sports during the 2-day experiment. Within 1 month after tape stripping, the wound healing was evaluated in a second dermatological examination. Fig. 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 consent for participation.

Application of the formulations

Three skin sites per arm were treated (total of six skin sites per volunteer). The application area was delineated by a rectangular glass frame (10.5 cm^2) glued onto the skin (Sauer skin glue, Manfred Sauer GmbH, Lobbach, Germany). A volume of 250 μl formulation was uniformly applied with a Hamilton Syringe (Supelco, Buchs, Switzerland), and the vehicle was allowed to evaporate. In Experiment 1, the high TACA dose (300 μg/cm^2) and the low TACA dose (100 μg/cm^2) were applied all at the same time (Time 0 h, at 9 a.m.) on three different sites per arm. In Experiment 2, the high TACA dose was applied at once on one arm (at 9 a.m.), whereas the low TACA dose was applied thrice on the other arm (at 9 a.m., 1 p.m., and 5 p.m.) (Fig. 1). Skin sites not stripped within 0.5 h after application were covered with non-occlusive cotton gauzes until tape stripping. No skin washing was performed to remove potential excess of formulation, because washing procedures have been correlated with an enhanced percutaneous absorption [29].

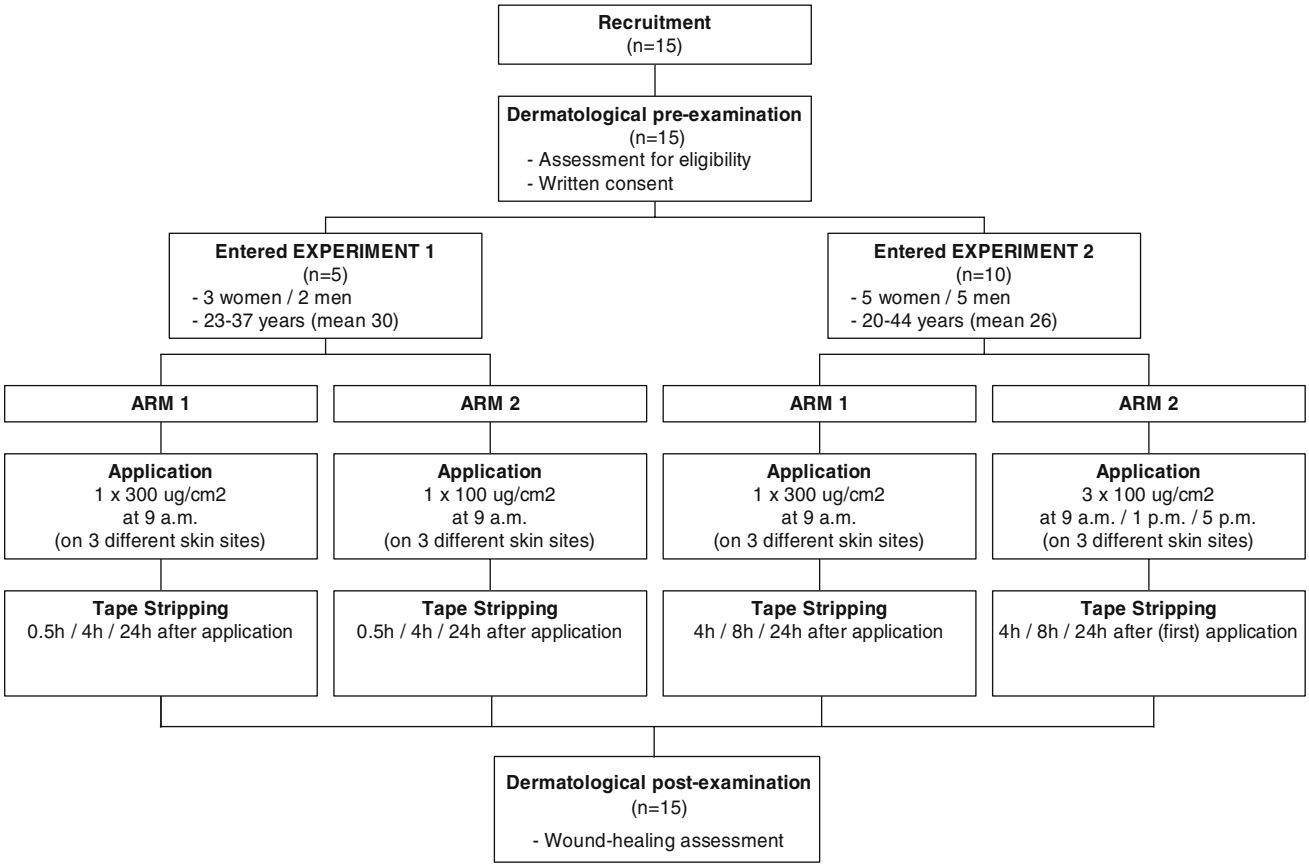

Fig. 1 Flow chart of the study: from recruitment to dismissal of the volunteers

Skin sampling by tape stripping

Stratum corneum tape stripping was performed after the following time intervals: in experiment 1 at 0.5, 4, and 24 h after application; in Experiment 2 at 4, 8, and 24 h after (first) application (Fig. 1). Following this schedule, the skin sites treated by multiple application in Experiment 2 are stripped 0.5 h after the second (time 4 h) and third applications (time 8 h), and 24 h after the first application.

To remove the stratum corneum in a standardized manner from the exact same skin area, a template delineating a constant aperture of 3.3×1.7 cm (5.6 cm^2) was fixed on the skin. An adhesive tape (Tesa Multi-Film Crystal-Clear® 57315, 19 mm width, Tesa, Beiersdorf, Hamburg, Germany) was placed on this skin site, and a hand roller supplying a pressure of 140 g/cm^2 was passed over the tape ten times. The tape was removed with a rapid, firm movement and fixed across a photographic slide frame. This procedure was repeated with new tapes until total removal of the stratum corneum, which was defined as light transmission through the tape $\geq 95\%$ (measured by UV/VIS spectroscopy, see next section). Tape stripping of one skin site lasted about 15 min. No significant further drug diffusion into deeper skin layers is expected during the tape stripping time, since the highest amount of drug is removed with the first tapes [13].

Quantification of corneocytes

The amount of corneocytes adhering to each tape was quantified by measuring the pseudo-absorbance of the corneocytes at 430 nm as described by Weigmann et al. [24]. The slide frames on which the tapes had been fixed were inserted in the sample holder of a Lambda 35 spectrophotometer (Perkin Elmer, Ueberlingen, Germany, custom-modified to obtain a rectangular light beam of 1 cm^2), and each tape was directly measured against a blank tape.

Quantification of TACA

After quantification of the corneocytes, each tape was disassembled from the frame and extracted with 1.5 ml 60% methanol during 30 min on a horizontal

shaker at 140 rpm (Heidolph Unimax 2010, Heidolph, Germany). To allow the calculation of a mass balance, the gauzes used to cover the treated skin sites were extracted similarly with 10 ml 60% methanol. TACA amounts in the extracts were quantified by an ICH-validated HPLC method [8] using a Symmetry Shield™ RP18 column (2.1 × 100 mm, 3.5 µm particle size) and a Waters Alliance HPLC System (2690 Separation Module, 996 Photodiode Array Detector), all Waters Corporation, Millford, Massachusetts, USA. The mobile phase consisted of methanol 60% in water (v/v) with a flow rate of 0.3 ml/min. Sample aliquots of 20 µl were injected, and quantification occurred at 240 nm. The limit of quantification (LOQ) was 100 ng/ml (corresponding to 27 ng/cm^2), the limit of detection (LOD) 35 ng/ml (9 ng/cm^2).

Data analysis

Sample size

The method deviation (intra-individual standard deviation) had been determined previously and was ± 40%. To provide a power of 80% in detecting a 50% difference between the two treatments groups at the 5% significance level, a total number of ten volunteers are needed according to the two-sided *t*-test nomogram for paired values after logarithmic transformation [21], and were thus enrolled in the main Experiment 2. For the explorative Experiment 1, a power of 50% was accepted, implicating the enrolment of five volunteers.

Qualitative TACA penetration into stratum corneum (penetration profiles)

To graphically visualize the drug distribution within the stratum corneum, TACA amounts quantified on each tape were correlated with the tape number and depth of penetration into stratum corneum. Removal of the entire stratum corneum is a prerequisite for the profile calculation, since the sum of corneocytes (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. [9, 26].

Quantitative TACA penetration into stratum corneum

The TACA amounts on each tape (area 5.6 cm^2) of each skin site were added up to the total TACA amount penetrated into stratum corneum, which was evaluated statistically. The significance of differences between the treatment groups at each time was tested in a two-sided paired-sample *t*-test after logarithmic transformation. Two different types of evaluation were performed: the evaluation of (a) the total TACA amount within 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, Maryland, USA) was used to conduct the analysis of the trial.

Mass balance

To gain further information on the fate of TACA, a mass balance was performed. The following TACA amounts were calculated: (a) TACA in the gauzes used to protect the application sites until tape stripping, (b) TACA in tapes 1–3, (c) TACA in the stratum corneum (without tapes 1–3), and (d) TACA not recovered and presumably penetrated into deeper skin layers or diffused laterally.

Results

Demography of the subjects

A total of 15 healthy adult volunteers (seven males and eight females) aged 20–44 years (mean 27) were recruited and completed the study. Five volunteers were assigned to Experiment 1 and ten volunteers to Experiment 2 (Fig. 1). The tape stripping experiments were conducted from March 2004 to July 2004 at the University Hospital 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.

Qualitative TACA penetration into stratum corneum (penetration profiles)

The depiction of the results as penetration profiles visualizes the localization of TACA within the stratum corneum. The typical penetration profile displayed large TACA amounts in the upper stratum corneum and lower TACA amounts in the deeper stratum corneum, indicating TACA permeation through the stratum corneum and penetration into deeper tissues. In both experiments, similar penetration profiles of TACA over time were achieved, apart from a higher TACA amount usually located on the first stripped tape after application of the

higher dose (300 μg/cm^2) in Experiment 1 or after multiple application (3 × 100 μg/cm^2) in Experiment 2. The penetration profiles obtained after the different applications of Experiment 2 in one volunteer are displayed in Fig. 2.

The mean number of tapes required to remove the entire stratum corneum in both experiments was 55, with a minimum of 29 tapes and a maximum of 80 (independently of gender and age).

Quantitative TACA penetration into stratum corneum

Experiment 1: Effect of dose (1 × 300 μg/cm^2 vs. 1 × 100 μg/cm^2)

The total TACA amounts penetrated into stratum corneum at the different time points are depicted in Fig. 3 (left side). At 0.5 h, almost the entire TACA dose applied was quantified within the stratum

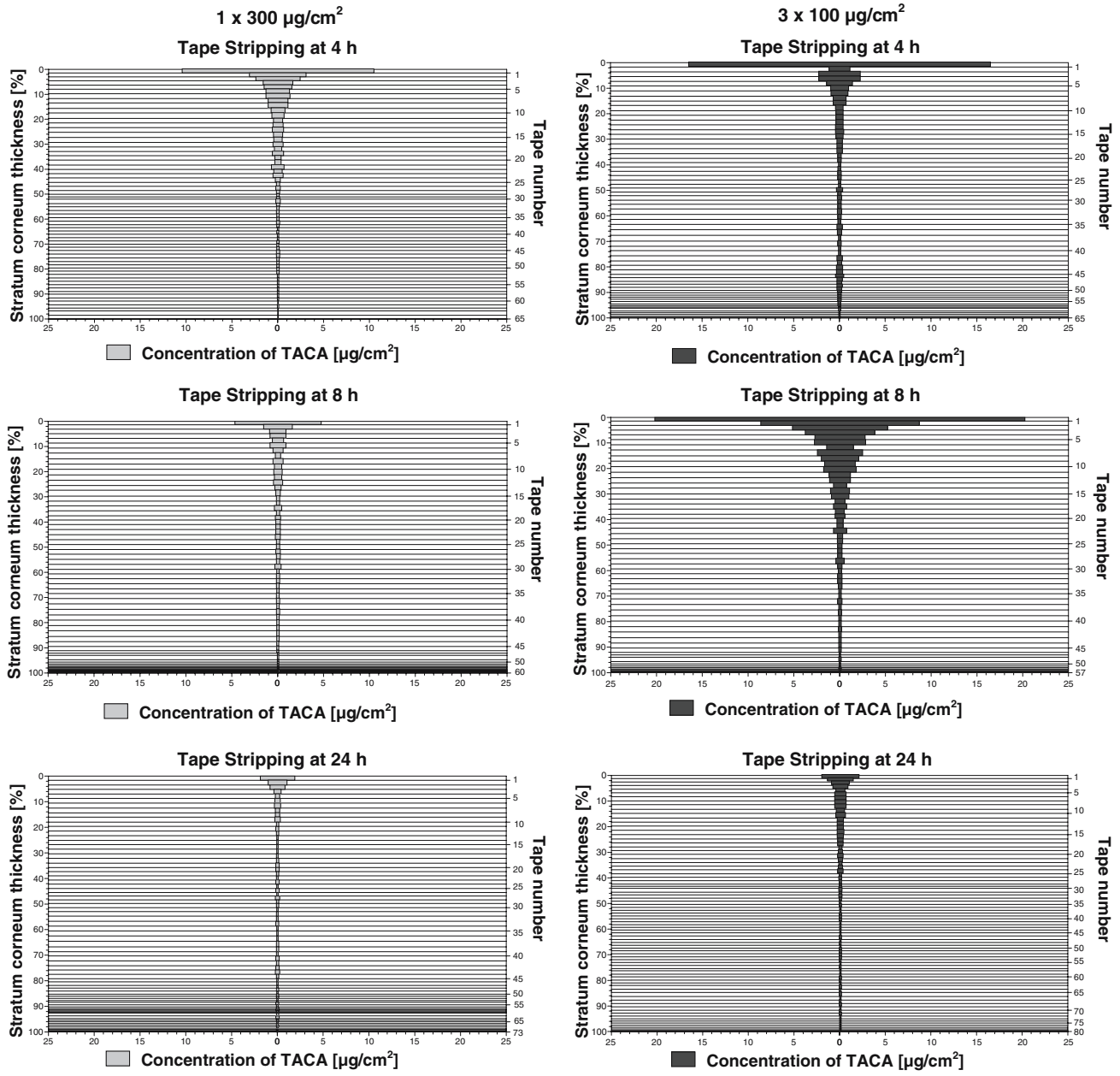

Fig. 2 Typical penetration profiles of TACA into stratum corneum during time (4, 8, 24 h) after application of 1×300 μg/cm^2 vs. 3×100 μg/cm^2 TACA in the same volunteer (Experiment 2). TACA amounts on each tape (*horizontal grey bars*) are correlated to the tape number (*right scale*) and to the depth of penetration into stratum corneum, displayed as percentage of the total stratum corneum thickness (*left scale*). This correlation is enabled because the entire stratum corneum was stripped from each skin site, the total number of tapes thus representing 100% stratum corneum thickness

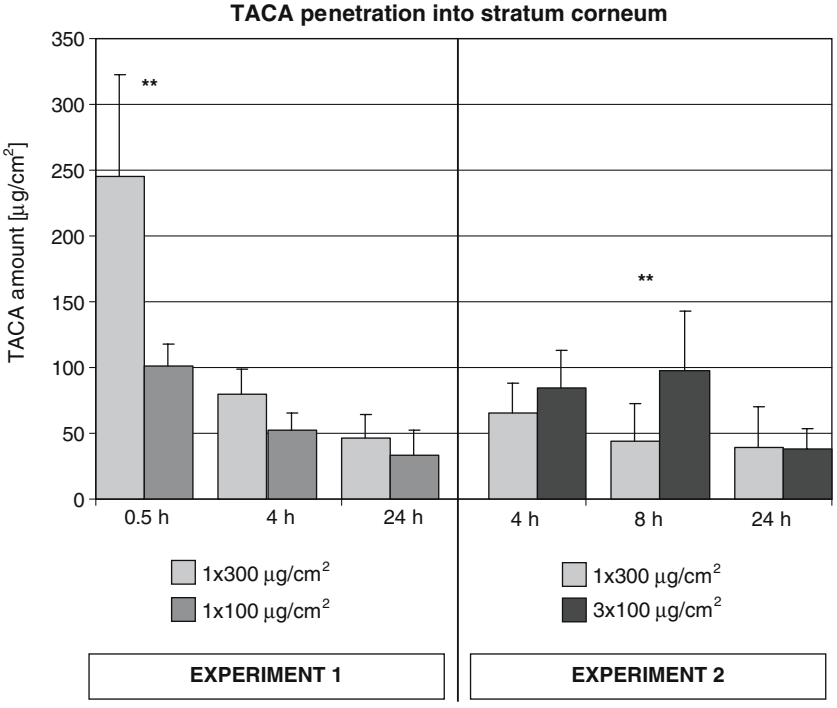

Fig. 3 Total TACA amounts penetrated into the stratum corneum in Experiment 1 and Experiment 2. Mean and standard deviation calculated with all tapes (including tapes 1–3) are displayed. The *double asterisk* (**) denotes a highly significant difference ($P < 0.01$, 2-sided paired-sample *t*-test) between the pair differences at the specified time

corneum: 245 ± 78 µg/cm^2 after application of 300 µg/cm^2 and 101 ± 17 µg/cm^2 after application of 100 µg/cm^2. At 4 h, lower TACA amounts of 80 ± 19 µg/cm^2 (after application of 300 µg/cm^2) and 52 ± 13 µg/cm^2 (after application of 100 µg/cm^2) were observed, and after 24 h still 46 ± 18 µg/cm^2 vs. 33 ±19 µg/cm^2 were quantified.

By excluding tapes 1–3, a dramatically lower amount was measured at 0.5 h within the stratum corneum, with TACA amounts of 74 ± 12 µg/cm^2 (after application of 300 µg/cm^2) vs. 55 ± 14 µg/cm^2 (after application of 100 µg/cm^2). No difference in the TACA amounts recovered after application of the two different doses was observed at later time points: 31 ± 15 µg/cm^2 vs. 30 ± 16 µg/cm^2 at 4 h, and 27 ± 13 µg/cm^2 vs. 23 ± 14 µg/cm^2 at 24 h after application of 300 µg/cm^2 and 100 µg/cm^2, respectively. The extremely lower TACA amount obtained by exclusion of tapes 1–3 showed that considerable amounts remained on the skin surface.

Statistical evaluation of the corresponding pair differences yielded a highly significant difference of the TACA amounts quantified at 0.5 h when all tapes were considered ($P < 0.01$) but no significance when tapes 1–3 were excluded ($P > 0.05$). At 4 and 24 h, no statistically significant difference was observed for both evaluations (all tapes/without tapes 1–3).

Experiment 2: Effect of application frequency (1 × 300 µg/cm^2 vs. 3 × 100 µg/cm^2)

The total TACA amounts penetrated into stratum corneum at the different time points are depicted in Fig. 3 (right side). At 4 h, the total TACA amount quantified within the stratum corneum (all tapes) amounted to 65 ± 23 µg/cm^2 after application of 1 × 300 µg/cm^2 vs. 84 ± 29 µg/cm^2 after 2 × 100 µg/cm^2. This slight difference became inexistent after discarding the first 3 tapes (33 ± 12 µg/cm^2 and 35 ± 11 µg/cm^2, respectively). The pair difference was statistically not significant in both cases ($P > 0.05$). Note that this time point displays a different totally applied dose (300 µg/cm^2 vs. 200 µg/cm^2). Taking this into account, the amount observed after multiple application was quite high: despite the application of a lower total dose, a similar TACA amount was quantified within the stratum corneum.

At 8 h, TACA within the stratum corneum amounted to 44 ± 29 µg/cm^2 (after application of 1 × 300 µg/cm^2) vs. 98 ± 45 µg/cm^2 (after application of 3 × 100 µg/cm^2). This difference was highly significant ($P < 0.01$) when all tapes were considered, but only a slight trend was seen after discarding tapes 1–3 (20 ± 8 µg/cm^2 and 29 ± 15 µg/cm^2 after application of 1 × 300 µg/cm^2 and 3 × 100 µg/cm^2, respectively, $P = 0.06$).

At 24 h, similar total TACA amounts were quantified within stratum corneum: 39 ± 31 µg/cm^2 after application of 1×300 µg/cm^2 vs. 38 ± 16 µg/cm^2 after 3×100 µg/cm^2 (24 ± 14 µg/cm^2 and 23 ± 9 µg/cm^2, respectively, after discarding tapes 1–3). These values showed no statistically significant difference ($P > 0.05$).

Mass balance

The application of a finite TACA dose permits the performance of a mass balance. For each treated skin site, 4 different values of recovered TACA amount were determined: (a) in the gauze, (b) in tapes 1–3, (c) in the stratum corneum without tapes 1–3 and, (d) the remnant amount not recovered and presumably penetrated into deeper tissues or diffused laterally. The results of the mass balance are presented in Fig. 4 (Experiment 1) and Fig. 5 (Experiment 2). The evaluation of both experiments showed that: (a) TACA amounts of 10–23 µg/cm^2 did not penetrate into the stratum corneum and adhered to the gauze (corresponding to 5–12% of the applied TACA dose), (b) About half the amount recovered in the entire stratum corneum persisted within the upper stratum corneum and was found in tapes 1–3 (up to 57% of the applied TACA dose), (c) TACA amounts recovered in the stratum corneum without tapes 1–3 were not significantly different between the different application modes, (d) TACA seemed to permeate the stratum corneum more rapidly after a single application of the high TACA dose (300 µg/cm^2), since the TACA amounts not recovered (and presumably penetrated into deeper tissues) were already observed after 0.5 h (Experiment 1) and were high after 4 h (in both Experiments 1 and 2).

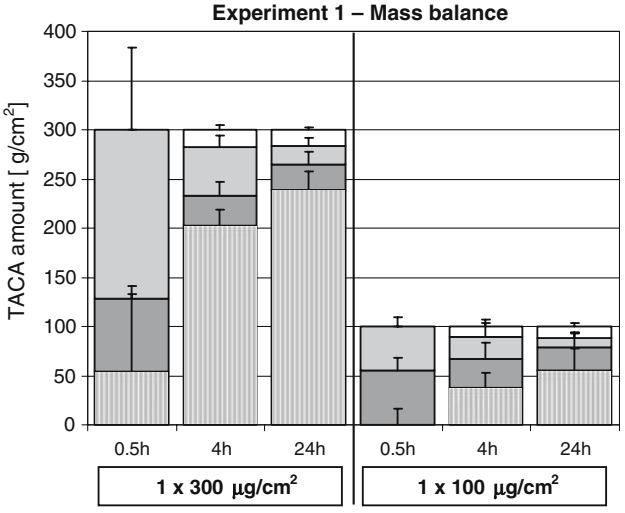

Fig. 4 Mass balance of Experiment 1. Skin sites are divided into four compartments displaying the mean TACA amounts (incl. standard deviation) [µg/cm^2] recovered in gauze (*white bars*), in tapes 1–3 (*light grey*), in stratum corneum without tapes 1–3 (*dark grey*), and the amounts of TACA not recovered and presumably penetrated into deeper or adjacent skin tissues (*striped grey*). At 0.5 h, no gauze was used, since tape stripping followed just after application of the formulation

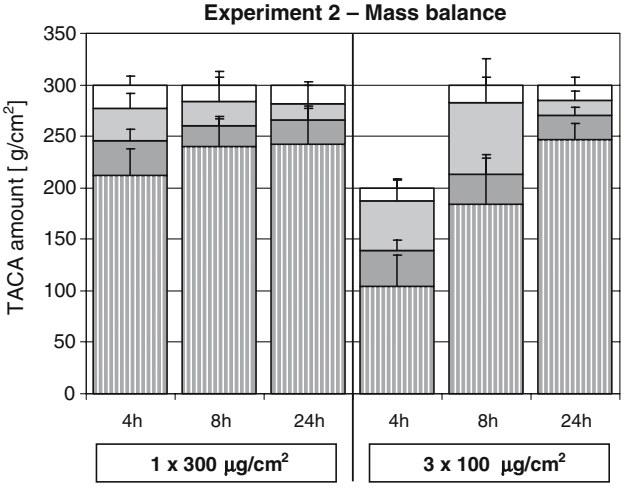

Fig. 5 Mass balance of Experiment 2. Each skin site is divided into four compartments displaying the mean TACA amounts (incl. standard deviation) [µg/cm^2] recovered in gauze (*white bars*), in tapes 1–3 (*light grey*), in stratum corneum without tapes 1–3 (*dark grey*), and the amounts of TACA not recovered and presumably penetrated in deeper or adjacent skin tissues (*striped grey*)

Discussion

A prerequisite for investigating topical products is the stringent differentiation between topical and systemic bioavailability determination. In previous investigations on percutaneous absorption of corticosteroids applied in acetone [11, 27], conclusions for topical therapy were drawn from data obtained by urinary excretion. Yet, data yielded from urinary excretion measure systemic bioavailability (of the topical application) and not topical bioavailability (of the topical application). Measurements of drug concentration in urine and/or blood are only a means for bioavailability evaluation when the pharmacological response is correlated to a systemic parameter or when the body burden is of interest. Furthermore, systemic drug concentrations after topical application do not represent drug concentrations at the target site in the skin but purely drug concentrations after permeation through the target site. Thus, the mere assessment of systemic bioavailability does not properly reflect topical bioavailability for the treatment of local skin diseases [3, 10].

This trial has been performed to assess the topical bioavailability of TACA in a simple vehicle after different application modes. Acetone does not represent a vehicle normally used in dermatology, but has often been used as a vehicle for investigative purposes [4, 11, 27]. TACA displays a good solubility in acetone, and the volatile vehicle allows the application of a finite, solvent-deposited drug amount [2, 16]. Therefore, the acetonic vehicle was appropriate for the purpose of this study. After application of the high TACA dose, the drug partially crystallized on the skin surface because of the rapid vehicle evaporation. On the one hand, the crystals can get lost due to friction with clothes or due to normal desquamation (this amount was retained and quantified in the protective gauze during our experiments). On the other hand, drug crystals can also become bioavailable at later stages if redissolved, either physiologically by humid micro environmental conditions on the skin surface or by fresh vehicle in case of multiple applications [18]. This was one reason for not washing the skin during our experiments, assuming that the protective gauze would retain the superficial, unbound TACA. Moreover, washing procedures have been shown to enhance percutaneous penetration of topically applied compounds [29].

A frequently debated question arising from tape stripping experiments is the inclusion or exclusion of the first tapes into the evaluation. The answer depends on the study design. In our case, the drug was solvent-deposited on the skin, and the mass balance required the consideration of all the tapes. Thus, a separate evaluation with and without tapes 1–3 was chosen.

In our experiments, the topical bioavailability of TACA was described by the TACA penetration into stratum corneum over time. This is possible because the stratum corneum is the rate limiting barrier to percutaneous absorption, and thus the amount of drug in the stratum corneum may reflect the amount of drug at the target site [17]. Experiment 1 displayed higher TACA amounts within the stratum corneum after application of a high TACA dose (300 $\mu g/cm^2$) compared to a lower TACA dose (100 $\mu g/cm^2$). However, this difference was only significant immediately after application, when almost the entire TACA dose applied was recovered within the stratum corneum, whereas similar TACA amounts were found after discarding tapes 1–3. Usually, increasing the applied drug dose leads to a higher absolute (but a lower relative) percutaneous penetration, provided that the drug is dissolved [30]. In our experiment, after application of the higher TACA dose, the immediate evaporation of acetone led to the precipitation of a high TACA amount on the skin surface and on the external layers of the stratum corneum.

Experiment 2 showed slightly higher TACA amounts within the stratum corneum after multiple application of a lower TACA dose (3×100 $\mu g/cm^2$) compared to the single application of the high TACA dose (1×300 $\mu g/cm^2$). As a result of multiple applications, the skin was periodically reloaded with new drug, thus achieving temporary higher amounts within the stratum corneum. In order to characterize this temporary trend, the stripping times after application of the second (at 4 h) and third (at 8 h) dose in case of multiple application were deliberately chosen. The highest drug amount was always localized within the upper stratum corneum layers and, by excluding tapes 1–3, only insignificant differences between the TACA amounts recovered after the different application modes were observed (in both experiments).

At 24 h, still well quantifiable TACA amounts were retained within the stratum corneum. This points out the property of stratum corneum to store topical applied corticosteroids, forming a reservoir. The term "reservoir" referred to the skin can be defined as an accumulation of a topically applied compound within the skin or within a particular skin layer for a longer time period. There are two main explanations for this accumulation. First, the accumulation can be due to a high partitioning of the compound into a specific skin compartment (e.g., into the intercellular lipids of the stratum corneum) and subsequent slow release into a deeper compartment. In this case, a low diffusional flux into deeper tissues is usually measurable over time [5, 15]. Second, a temporary sequestration of compound can occur because of binding to specific skin structures (e.g., to keratin, proteins, amino acids [23], collagen fibers [14], stratum corneum lipids [12]). Therefore, diffusion into deeper compartments may be discontinued for a longer time period. In the present experimental setting, the amount of TACA measured within stratum corneum decreased over time, showing that diffusion into deeper compartments, albeit slow, was taking place and long-term binding to specific skin structures was unlikely. The extent of the stratum corneum reservoir seemed to be independent from the dose applied and the application frequency, because the TACA amounts measured after 24 h were similar in both experiments using this vehicle.

A similar topical bioavailability within the stratum corneum does not necessarily imply a similar systemic bioavailability, not desired in topical therapy with corticosteroids. TACA amounts not quantified within the stratum corneum have presumably penetrated vertically into the viable epidermis and into the dermis,

reaching the systemic blood circulation. In addition, a horizontal, lateral diffusion into adjacent stratum corneum has also been observed in the past [25]. Both penetration routes may have played a role in our experiments, but the vertical penetration represents the usually preferred route.

The mass balance was performed to estimate the extent of the systemic exposure. After application of a total TACA dose of 300 μg/cm^2 (either as a single dose or divided into multiple doses), a high TACA amount was not recovered and seemed to have penetrated into deeper tissues. At 24 h, the extent of percutaneous absorption and thus the extent of systemic bioavailability seemed independent of the application mode, but the release rate from the stratum corneum into deeper tissues was lower after application of the multiple doses. On the contrary, after application of a total dose of 100 μg/cm^2, both extent and release of the drug out of the stratum corneum into deeper tissues were lower. Thus, a low TACA dose applied once may be preferable to a higher total TACA dose (applied once or thrice daily). This is in agreement with pharmacodynamic investigations [7], which showed that multiple-daily applications usually have no superiority.

Conclusions

In the present study, the effect of dose and application frequency on the topical (cutaneous) bioavailability of TACA was determined by standardized tape stripping in human volunteers. Actual drug amounts were measured directly within the stratum corneum, a layer preceding the target site.

Independently of the dose and the application frequency, still well quantifiable TACA amounts were retained for 24 h within the stratum corneum (reservoir function). A difference between the TACA amounts that penetrated into the stratum corneum after the different application modes was observed immediately after application, and was mainly due to a different TACA amount in the first three tapes. No major differences were observed at later time points. Yet, a faster permeation through the stratum corneum and thus a higher systemic exposure, not welcome in topical therapy, may be promoted by application of a high TACA dose. Thus, a low TACA dose applied once may be preferable to a high TACA dose.

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