

INVESTIGATIVE REPORT

Impact of Adult Atopic Dermatitis on Topical Drug Penetration: Assessment by Cutaneous Microdialysis and Tape Stripping

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Appropriate methodologies for the determination of drug penetration in diseased skin have not yet been established. The aim of this study was to determine the cutaneous penetration of a metronidazole cream formulation in atopic dermatitis, employing dermal microdialysis and tape strip sampling techniques. Non-invasive measuring methods were used for the quantification of the severity of the dermatitis. Skin thickness and the depth of the microdialysis probes in the skin were measured by 20 MHz ultrasound scanning. Metronidazole concentration, sampled by microdialysis, was 2.4-fold higher in the atopic dermatitis compared with uninvolved skin ($p < 0.001$). Tape stripping methodology did not disclose this difference in penetration. Thus, the skin layer of interest and the integrity of the skin barrier should be considered when selecting sampling methodology. Microdialysis sampling is the method of choice whenever the dermis is the target tissue for topical treatment and a skin disease affecting the barrier function is present. Key words: atopic dermatitis; dermatopharmacokinetics; metronidazole; microdialysis; skin penetration; tape stripping; topical formulations.

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Atopic dermatitis (AD) is a frequent skin disease with a biological skin barrier defect determined by genetic factors. The clinical picture of AD varies over the years and it is often limited to childhood. In a proportion of patients the disease lasts into adulthood (1).

For treatment of this skin disease the first choice is a topical corticosteroid, accompanied by antibiotics or antimycotics whenever an infection is present.

Few techniques are available for the assessment of *in vivo* topical drug penetration in human skin. The bioavailability and potency of topical corticosteroids can be investigated by the skin blanching technique (2). Other methods, such as punch biopsies, suction blisters and shave biopsies, can be useful for the determination

of cutaneous drug penetration but have no place in human studies due to their invasive nature and the limited information provided.

Recently two methods have been evaluated for the investigation of drug penetration from topical formulations: microdialysis (MD) sampling and tape stripping (TS) methodology (3, 4). MD is a technique for *in vivo* sampling of endogenous and exogenous substances in the extracellular fluid, which represents up to 20% of the tissue volume. The technique has developed into a valuable tool for pharmacokinetic, pharmacodynamic and bioequivalence studies in human skin (5–11). For a more detailed description of this technique see Groth et al. (7)

TS of the skin relies on sampling of the stratum corneum (SC) cells by successive tape application and removal. This is conducted a short time (usually 30 min) after topical drug application and following removal of excess formulation. The drug content in the SC material, collected by ten consecutive tape strips, is then analysed (yielding a single end-point measurement). The percutaneous absorption of the compound over the following days has been predicted by linear extrapolation (12). TS, also called the dermatopharmacokinetic method, has previously been considered the method of choice for studies of topical drug penetration by the US Food and Drug Administration (2).

However, the usefulness of these two methodologies for the determination of topical drug penetration in the presence of skin disease is not known. Both methods have been evaluated in experimentally induced irritant dermatitis as a model for endogenous skin disease (4).

The aims of this study were: (i) to investigate whether MD and TS can be used for bioavailability studies in skin with AD; (ii) to determine if the methods would be correlated when sampling in diseased skin; (iii) to study the correlation between the severity of the skin disease and the drug concentration obtained by either technique; and (iv) to evaluate the variability obtained by both methodologies in the presence of endogenous skin disease.

The formulation chosen for the investigation was metronidazole in a cream formulation (Flagyl® 1% cream), since this drug is accessible to sampling by both methods and the drug concentration in the samples can be analysed by high-performance liquid chromatography and mass spectrometry (HPLC-MS) with a very low limit of quantification (4).

MATERIALS AND METHODS

The study was approved by the Copenhagen County ethics committee (ref. KA 05032) and was undertaken in accordance with the Declaration of Helsinki. Subjects were given a detailed description of the study and their written consent was obtained.

Study population

Six subjects with adult AD, 5 men and one woman, with a mean age ($\pm$ standard deviation (SD)) of 30 ± 9 (range: 19–45 years) participated in the study. All had active AD on the skin of the volar aspect of both forearms. The volunteers did not have any disease (skin or systemic) other than the AD, and they refrained from taking medication for 2 weeks prior to the study. Topical moisturizers were allowed up to 12 h prior to the experimental day. The severity and duration of flare-up of their eczema varied from erythema with scattered papules, to more chronic changes represented by lichenification, dryness and scaling of the skin (see Fig. 1 as an example).

Study formulation

Flagyl® cream 1% (Aventis Pharma A/S, Hørsholm, Denmark) was used as is. For the local anaesthesia prior to the insertion of the probes, subcutaneous injections of lidocaine (Xylocaine® 10 mg/ml, AstraZeneca, Albertslund, Denmark) were used. The perfusate for the MD experiment consisted of sterile isotonic saline solution (sodium chloride 9 g/l, 308 mmol/l).

Experimental protocol

At the beginning of the experimental day non-invasive measurements (transepidermal water loss (TEWL) and erythema) of barrier integrity were performed and followed by a standardized gentle wash of both forearms. The penetration areas were demarcated on the skin by drawing with a permanent marker pen: two areas measuring 2×2.6 cm on the left forearm (one with changes from the AD, and one with uninvolved skin) for Dermal Microdialysis (DMD) sampling; on the right forearm four round areas with a diameter of 2.2, two in uninvolved skin and two in skin with AD for TS harvesting (Figs 1 and 2). A control area was placed above the wrist and stripped at the beginning of the experiment to establish a baseline of drug content in the skin. The content was below the limit of detection (0.2 ng/ml of the final sample solution) in all tape strip control samples.



Fig. 1. Application area for microdialysis sampling. Area 2×2.6 cm with atopic dermatitis on the left forearm.

In vivo microdialysis

Local anaesthesia was injected in the dermis/subcutaneous tissue around the demarcated areas (volume < 20 ml), and two linear MD probes were inserted in each area parallel to each other and to the skin surface by means of a 22G guide cannula. The outlet was placed in a 300- μ l glass vial, and perfusion was started at a low flow (0.4 μ l/min). MD sampling at a 1.173 μ l/min flow was started following an equilibration period of 1 h after probe insertion, in order to allow the insertion trauma to subside, as shown necessary by Groth & Serup (13). Following a 20-min baseline sample the formulation was applied to the skin above the two investigational areas and samples were collected every 20 min for 3 h. The length of the membrane accessible to microdialysis was 3 cm in all experiments. From a previous study (García Ortiz et al., submitted to Skin Pharmacology), the *in vivo* relative recovery (RR), determined by *in vivo* loss, of metronidazole with the present experimental set-up was known to be 34%.

In vivo tape stripping

The metronidazole (MTZ) formulation was applied to the demarcated areas on the right forearm for TS harvesting at the same time as application to the MD locations. Thirty minutes after application two of the penetration areas were stripped (one in uninvolved skin, and one in skin with AD), and 120 min later the other two areas were harvested. D-squame® tapes with a diameter of 22 mm (CuDerm®, Texas, USA) were used. The skin surface was wiped twice with cotton gauze to remove residual formulation. Tape discs were applied and removed by pincers, using gentle pressure with the blunt end after application to assure good skin contact, and alternating strip removal directions (N, S, E and W). The first two discs were discarded, discs 3–12 stacked and placed in an airtight glass container and stored at -35°C until analysis.

Drug application

Flagyl® cream was applied at $t = 0$ to the 6 investigational areas of both forearms, using pre-weighed glass spatulas with a pre-weighed (Mettler Toledo PB303) amount of formulation 3 mg in excess of the intended dose; a dose of 4 mg/cm² was applied (as per AAPS/FDA Workshop Report, 1998 (2)) directly and with no occlusion to the skin surface.



Fig. 2. Application area for tape stripping. Round areas on the right forearm with atopic dermatitis for harvesting after 30 and 120 min (S30 and S120, respectively).

Non-invasive measurements of skin barrier function, erythema, skin thickness and probe depth

To establish the severity of barrier impairment in the presence of AD compared with the uninvolved skin of the patients, TEWL and erythema were measured at the beginning of the experimental day. TEWL measurements were performed by an evaporimeter (Dermalab Cortex technology, Hadsund, Denmark), according to guidelines (14) and recorded in triplicate. A colorimeter (Minolta Chromameter® CR300, Osaka, Japan) was used to measure erythema (the a^* value) (15) in accordance with the standardized Commission Internationale de l'Eclairage Guidelines.

At the end of the experiment the depth of the microdialysis probes in the skin and the skin thickness of the volunteers were measured by 20 MHz ultrasound scanning (Dermascan-C, Cortex, Hadsund, Denmark) in three separate scans along the length of the probe *in situ* (near probe entry, middle and near probe exit).

Sample analysis

The concentration of MTZ in dialysates was measured, without pre-treatment, by HPLC-MS. The content of MTZ in the tape strips was analysed following extraction procedures by LC-MS. For a detailed description of the analysis method see García Ortiz et al. (4).

Data analysis

All data are presented as means $\pm$ SD or standard error of mean (SEM). Logarithmic transformation of the results was required for the statistical analysis to achieve normalization and stabilize the variance of the data. This was checked graphically. The area under the concentration-vs.-time curve (AUC) was used for the pharmacokinetic analysis of MTZ dermal concentration in both normal and diseased skin. The statistical significance of differences in MTZ penetration between normal skin and AD was analysed using one-way analyses of variance (ANOVA) followed by pair-wise comparisons. The relationship between both methods in both situations was studied by linear regression analysis. A p -value < 0.05 was considered significant.

RESULTS

Metronidazole penetration by dermal microdialysis

The dermal concentration of MTZ sampled by MD was 2.4-fold higher in AD (671.7 ± 127.9 ng/ μ l $\times$ min) (mean $\pm$ SEM) than in uninvolved skin (285.5 ± 66.3 ng/ μ l $\times$ min), $p = 0.004$ (see Fig. 3 and Table I).

The intra-subject coefficient of variation in normal skin was 26%, and 83% in eczematous skin, whereas the inter-subject coefficient of variation (CV) was 264% and 120%, respectively.

Metronidazole penetration by tape stripping methodology

Results obtained by tape strip harvesting are shown in Fig. 4 and Table II. The content of MTZ in the SC sampled by this technique was approximately 15% lower in diseased skin (30 min: 34.3 ± 13.0 ; 120 min: 24.3 ± 6.4) (mean $\pm$ SD) than in uninvolved skin (30 min: 39.3 ± 11.0 ; 120 min: 29.7 ± 11.9), $p = 0.334$.

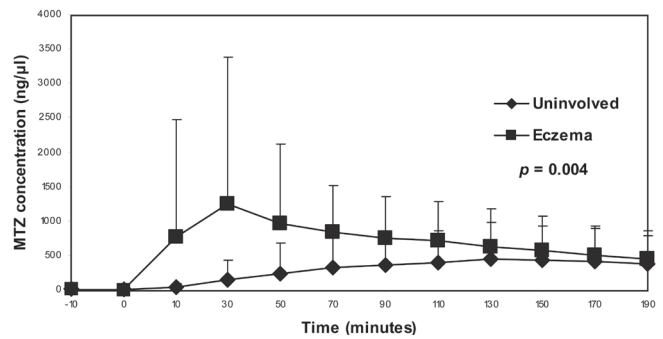


Fig. 3. Microdialysis. Concentration vs. time curve for the cutaneous penetration of metronidazole (MTZ). Mean concentrations are presented for $n = 6$. The area under the time vs. concentration curve was significantly larger in atopic dermatitis (eczema) compared with uninvolved skin ($p = 0.004$).

The inter-individual CV of TS was 18.6% in uninvolved skin and 25.8% in skin with AD.

Measurements of probe depth and skin thickness

The MD probes were all placed inside the dermis and approximately at the same depth in both uninvolved skin (0.79 mm $\pm$ 0.001) (mean $\pm$ SD) and skin with AD (0.86 mm $\pm$ 0.06), as measured by high-frequency ultrasound scanning, $p = 0.64$ (Table I).

The thickness of the skin of the volunteers was measured at the locations of MD sampling. Involved skin with AD was found to be significantly 0.12 mm thicker (equal to an increase of 8%) than uninvolved skin ($p = 0.015$).

Non-invasive measuring methods of skin barrier function and erythema

Both TEWL and erythema measurements significantly ($p < 0.05$) confirmed the barrier impairment and increased redness due to skin inflammation in skin with active dermatitis (Tables I and II). One exception was the measurement of erythema over the area for TS after 120 min, where the difference was not significant ($p = 0.11$).

Table I. Quantification of metronidazole penetration by microdialysis

Barrier perturbation	Uninvolved	Atopic dermatitis	p
Number of subjects	6	6	
Number of probes	12	12	
TEWL (g/m ² h) ^a	8.9 ± 5.5	30.6 ± 15.6	0.013
Erythema ^a	3.8 ± 1.3	8.3 ± 2.5	0.01
Skin thickness (mm) ^a	1.59 ± 0.1	1.71 ± 0.1	0.015
Probe depth (mm) ^a	0.79 ± 0.001	0.86 ± 0.06	0.6
AUC (ng/ μ l $\times$ min) ^b	285.5 ± 66.3	671.7 ± 127.9	0.004
95% CI (ng/ μ l $\times$ min)	(142.3; 428.7)	(395.7; 947.7)	

^aResults are expressed as mean $\pm$ standard deviation.

^bResults expressed as mean $\pm$ standard error of mean.

AUC: area under the time vs. concentration curve of the topical penetration of metronidazole; CI: confidence interval; TEWL: transepidermal water loss.

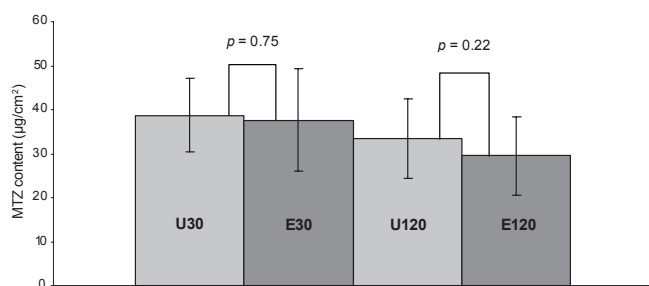


Fig. 4. Tape stripping. Metronidazole (MTZ) concentrations (mean $\pm$ SD) in tape strip samples of the stratum corneum from uninvolved skin (U30 and U120) and eczema (E30 and E120), after 30 and 120 min, respectively, $n=6$.

Here the redness was discrete, probably because in most volunteers the eczema was in a chronic phase.

Correlation between metronidazole concentrations and non-invasive bioengineering methods

For the left arm, where MD sampling was undertaken, we found the concentrations of MTZ sampled in uninvolved skin were positively correlated with their corresponding TEWL ($r=0.7$) and erythema ($r=0.69$) values, but these correlations were not significant. In areas with active eczema, no correlation between MTZ penetration and measurements of TEWL and erythema ($r=0.005$ and $r=-0.16$, respectively) was found.

For the right arm, where TS sampling was performed, no significant correlation between the content of MTZ and TEWL and erythema was found (neither for eczematous nor uninvolved skin).

DISCUSSION

This is the first study of topical drug penetration investigated in atopic dermatitis by dermal MD sampling and TS methodology.

Dermal MD demonstrated a 2.4-fold increased penetration of the MTZ topical formulation in active AD compared with uninvolved skin. The most authoritative

studies concerning topical drug penetration in patients with AD are those performed by Turpeinen and co-workers (16–18), in which the percutaneous absorption of hydrocortisone (HC) was investigated in 38 children by 4-h HC absorption tests. Here the cutaneous and percutaneous penetration of HC was significantly 2.6 times increased in the acute phase of AD, and this increase correlated significantly ($r_s=0.991$) with the severity of the skin disease as measured by TEWL. Similarly, the percutaneous absorption of HC during exacerbation and remission of AD in 18 adults and 16 children was investigated using the direct HC absorption test and 4-h HC absorption test, respectively (17, 18). During the convalescence period the percutaneous penetration measured by exogenous cortisol in plasma samples was 1/8 of that in the acute phase of the disease. This reduction can be attributed to the restoration of the skin barrier function and, as a consequence, reduced penetration of HC.

Working with irritant dermatitis as a model for endogenous skin disease and intradermal MD sampling of topical penetration, the cutaneous penetration of salicylic acid has been shown to be positively and significantly correlated with the degree of barrier impairment as measured by TEWL and erythema (3). In a recent study (4) our group have reported a significantly ($p<0.001$) three-fold increased penetration of a MTZ formulation sampled by dermal MD in skin with mild irritant dermatitis (sodium lauryl sulphate 1%) compared with normal skin.

In the present study we could not find a correlation between MTZ penetration and skin barrier function evaluated by TEWL and erythema measurements. The explanation probably lies with the modesty of the skin changes in the AD as well as the small study population.

In contrast to these findings, TS methodology yielded samples with reduced MTZ content from application of the formulation onto eczematous skin compared with uninvolved skin. However, the reduction of approximately 16% was not significant. In a recent *in vivo* TS study of percutaneous penetration in AD compared with normal skin, Jakasa et al. (19) showed an increased dif-

Table II. Quantification of metronidazole (MTZ) penetration by the tape stripping (TS) methodology

Barrier perturbation	Uninvolved skin		Atopic dermatitis	
	TS30min	TS120min	TS30min	TS120min
Number of subjects	6	6	6	6
TEWL (g/m ² h) ^a	9.5 $\pm$ 5.6	8.6 $\pm$ 3.6	27.3 $\pm$ 14.9	28.2 $\pm$ 17.4
Erythema ^a	4.3 $\pm$ 1.6	4.3 $\pm$ 1.7	7.6 $\pm$ 1.6	6.9 $\pm$ 2.1
Content (µg/cm ²) ^a	39.3 $\pm$ 11.0	29.7 $\pm$ 11.9	34.3 $\pm$ 13.0	24.3 $\pm$ 6.4
95% CI (µg/cm ²)	(29.6; 49.0)	(19.1; 40.3)	(22.8; 45.8)	(18.7; 29.9)

p-values concerning the comparison between uninvolved and eczematous skin:

Location: TS30 min		Location: TS120min	
MTZ concentration	0.75	MTZ concentration	0.22
TEWL	0.02	TEWL	0.03
Erythema	0.03	Erythema	0.11

^aResults are expressed as mean $\pm$ standard deviation.

TEWL: transepidermal water loss; CI: confidence interval.

fusion coefficient of polyethylene glycols of different molecular weight in both involved and uninvolved eczematous skin compared with normal skin. Thus, considering the findings of this recent publication as well as the consistency of the results from investigations referred to earlier, where dermal and systemic drug concentrations were increased in the presence of AD, it can be speculated that a faster initial penetration into and through the epidermis can account for the lower concentrations found in the SC. Furthermore, this explanation is corroborated by the dermal pharmacokinetics of MTZ penetration as sampled by MD (Fig. 3), where the maximum concentration of MTZ is found at 30 vs. 150 min, respectively. Despite this "direct evidence" of a faster MTZ penetration in AD, we admit that a follow-up experiment where TS was conducted at, for example, 5, 10, 15 and 20 min following drug application would further clarify our finding of reduced MTZ content in TS samples from eczematous skin.

The intra-individual variation of MD results in skin with AD (CV of 83%), was higher than in uninvolved skin (CV of 26%), possibly due to variability in the extent and severity of eczema across the application sites. The inter-individual variability was notably high both in eczema (120%) and normal skin (264%); differences in the severity of the AD of the volunteers as well as in the degree of the impairment of barrier function in the uninvolved skin must account for these results. Numerous publications (20–23) have shown an increased susceptibility of straightforward impaired barrier function of apparently uninvolved skin of subjects with AD compared with healthy subjects. Little variation was found in the results obtained by TS in both the AD (CV of 25.8%) and uninvolved skin (CV of 18.6%). This is concordant with our previous investigation of topical penetration in irritant dermatitis (4).

Hence, this is the first study of topical drug penetration where microdialysis and tape stripping methodologies have been used in skin with atopic dermatitis. This study has demonstrated that: (i) both sampling techniques are able to sample cutaneous drug penetration in the presence of skin disease. Even sparse AD resulted in a 2.4-fold increase in dermal MTZ concentration as measured by MD sampling, whereas TS method did not detect this increase in penetration. (ii) Consequently, the two methodologies did not correlate when sampling in diseased skin. (iii) An absence of correlations between the drug penetration sampled by both techniques and non-invasive measurements of skin barrier function was found. This could be explained by the fact that the eczema of the participants was in a chronic stage, thus giving only a modest increase in TEWL and erythema values, in combination with the small study population size. (iv) The presence of atopic eczema resulted in a doubling of inter-individual variability in MD results, whereas TS variability was largely unaffected.

In conclusion, dermal MD seems to be the most suitable technique for the study of topical drug penetration in atopic dermatitis. However, further investigations are needed for a more detailed characterization of the results obtained by this technique and their correlation with the measurements of the integrity of the skin by non-invasive methods.

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