Ex vivo Cutaneous Bioavailability of Topical Mometasone Furoate in an O/W Preparation

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Keywords

Mometasone furoate · Drug delivery · Cutaneous bioavailability · Penetration · Scratch assay

Abstract

Mometasone furoate (MMF) is a modern glucocorticoid of the 4th generation, which has been proven not only for inhalation but also for cutaneous treatment. Due to its lipophilic character, it is mainly used in ointments and creams with an outer lipophilic phase (W/O type). However, this study investigated the cutaneous cytotoxicology of MMF and tried to characterize its pharmacokinetic effects on the skin using an O/W preparation. An HPLC method has been developed and validated for the detection of MMF in cutaneous tissue, and concentration-time curves of MMF were created after cutaneous application on unaffected as well as lesional skin. Cytotoxicological characterization was carried out using scratch assays on keratinocytes and cutaneous fibroblasts. Results showed that the condition of the skin had no significant impact on the cutaneous bioavailability of MMF, but the intrinsic effect of the O/W vehicle could be utilized in periods of acute inflammation. Cytotoxicological data gave no new indications regarding the safety of MMF.

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Introduction

Mometasone furoate (MMF) is a synthetic halogenated monoester of prednisolone with high lipophilicity (octanol water partition coefficient [K_{OW}] of 2,150) and a high benefit-risk ratio [1–3]. Originally used as an inhaled glucocorticoid for respiratory diseases, subsequently MMF has also been used in lipophilic vehicles for epicutaneous application to treat inflammatory skin diseases [4–10]. MMF is freely soluble in acetone and methylene chloride, slightly soluble in ethanol, but practically insoluble in water. The drug is degraded into 6- β -hydroxymometasone by cytochrome P₄₅₀ 3A4 [11, 12]. Its pharmacokinetic properties are comparable with other glucocorticoids [8, 13–17]. MMF is classified by Niedner [18, 19] as a highly potent glucocorticoid (class III out of IV). The duration of application and the galenic properties of the vehicle have a major influence on the therapeutic effect [20–23]. The efficacy of MMF as a topical treatment has been proven for a variety of inflammatory dermatoses [24–40]. Based on data from dose-finding studies, MMF for topical application is used in semisolid preparations in a concentration of 0.1% [41].

In order to make better use of the intrinsic effect of the vehicle, especially in periods of acute inflammation, an

Table 1. Processing of the cutaneous layers and additional samples

Compartment	Sample	Section scheme	Extraction volume
Nonpermeated fraction	Swab		2.0 mL
Skin sections	Stratum corneum (SC)	10 μ m	0.1 mL ^a
	Vital epidermis (EP)	4 sections à 20 μ m	0.1 mL ^a /0.4 mL ^b
	Dermis 1 (DR 1)	5 sections à 40 μ m	0.1 mL
	Dermis 2 (DR 2)	5 sections à 40 μ m	0.1 mL
	Dermis 3 (DR 3)	5 sections à 40 μ m	0.1 mL
	Dermis 4 (DR 4)	5 sections à 40 μ m	0.1 mL
	Dermis 5 (DR 5)	5 sections à 40 μ m	0.1 mL
	Remaining stump		0.1 mL ^a /0.2 mL ^b
Permeated fraction	Filter gauze		2.0 mL
	Acceptor fluid		–/–

^a Intact skin. ^b Lesional skin.

MMF-containing O/W vehicle has recently been created, whose efficacy can be compared to conventional W/O cream or ointment preparations [21, 24]. With regard to a continuous long-term application especially on damaged skin, the concentration-time profile of MMF in the different skin layers is of vital importance not only for its therapeutic effect but also for the severity of its adverse reactions. Apart from the classical group effects within the genomic and nongenomic effects of glucocorticoids, direct substance-specific metabolic and cytotoxic effects have to be considered, which can affect mainly keratinocytes (epithelial atrophy) and fibroblasts (inhibition of collagen synthesis) as the main cell types in the respective target compartments [42, 43].

The aims of this study were to validate penetration profiles of the new galenic MMF preparation into intact as well as damaged skin and to prove its safety regarding the cytotoxicological effects of relevant MMF concentrations.

Materials and Methods

Analytic Method

An analytic HPLC-UV method for the quantification of MMF in skin was developed using an Agilent 1200 HPLC system (Agilent, Waldbronn, Germany) and validated according to FDA guidelines concerning selectivity, reproducibility, precision, and accuracy [44]. All samples were calculated using the method of external standard and weighted with $1/C^2$. MMF was determined using a ReproSil 100 C18, 5 μ m, 125 $\times$ 4 mm (Wicom, Heppenheim, Germany) with precolumn RP 18 EC 3/4 (Macherey-Nagel, Düren, Germany), a flow of 0.8 mL min⁻¹ acetonitrile/water (80/20

V/V) + 0.1% formic acid, 30°C column temperature, and 15 μ L injection volume. The detection was carried out by UV (240 nm). The method had a limit of determination of 0.064 μ g/mL and a limit of detection of 0.005 μ g/mL.

Sample Preparation

Sections from the same skin compartment (Table 1) were stored together at –20°C until processing and measuring. Swabs, which were used to absorb excess MMF, and filter gauzes were stored in 15-mL test tubes. Acetonitrile/water 80/20 (v/v) was added for extraction (Table 1), and the test tubes moved for 24 h on a laboratory shaker. Skin samples were afterwards centrifuged at 13,000 rpm for 10 min, and the supernatants were analyzed. One milliliter of the acceptor liquid from all cells, respectively, was evaporated under nitrogen atmosphere at 40°C and resolved with 0.5 mL acetonitrile/water 80/20 (v/v). The concentrated samples were measured. Extracts of the swabs were diluted (1:10). For safeguarding, quality controls with known concentrations were carried out in parallel.

Test Preparation

A hydrophilic cream (O/W type) containing 0.1% MMF (Monovo®; 1 mg/g creme; Almirall Hermal GmbH, Reinbek, Germany) was used.

Diffusion Tests

The tests were performed on excised human skin from reduction mammoplasty after proof of functional integrity as described previously [45] using a glass Franz diffusion cell (Crown Glass Company, Somerville, NJ, USA). Skin specimens of 3 donors each with 3 pieces of skin per setting and for each application period of 30, 100 and 300 min were investigated. For a second test series, the stratum corneum was manipulated with 10-fold stripping (Tesa®; Beiersdorf, Hamburg, Germany; size: 15 $\times$ 20 mm) before testing in order to simulate a barrier defect (lesional skin). To determine the amount of penetrated MMF for the respective application periods, the skin samples were removed from the diffusion cell and the residues of the preparation were carefully removed from the

surface using a cotton swab. In order to determine the concentration-time profile, the individual skin layers were horizontally separated using a cryomicrotome (stratum corneum: 1 section at 10 μm in intact skin experiments; vital epidermis: 4 sections at 20 μm ; corium: 25 sections at 40 μm + remaining stump). Demineralized water was used as acceptor. All samples were stored at -40°C until analysis.

Isolation, Cultivation, and Characterization of Native Cells from Human Skin

Keratinocytes and fibroblasts were isolated and cultivated from human foreskin according to standardized protocols. After treatment with dispase, native keratinocytes were isolated from the pre-isolated epidermis as well as cutaneous fibroblasts from the dermis [46, 47]. The cells were cultivated in 24-well microtiter plates (Greiner) at 37°C , 20% CO_2 , and 80% humidity until they reached a degree of confluency of 85% [47]. After cultivation, the cells were harvested using a cell harvester (Inotech, Wohlen, Switzerland) and kept in standardized aliquots.

Scratch Assay

Native cutaneous keratinocytes and fibroblasts of the 2nd passage were coated with laminin/fibronectin/collagen IV (4:50:1, v:v:v) in PBS (27.5:72.5, v:v), then seeded on 6-well plates (0.2×10^6 cells/well in 4 mL), and cultivated under the above-described conditions for 72 h until full confluency was reached [48]. For the simulation of a defect, a straight scratch was applied on the cell layer of each well using a sterile pipette tip. Subsequently, the cell debris was removed with pipettes, and a washing procedure with serum-free medium was carried out. DMSO (solvent control) and 0 (zero control), 0.5, 5.0, 50.0, and 100.0 $\mu\text{mol/L}$ MMF were added to the cultures, then incubated for 48 h, and photographed at 0, 2, 4, 8, 12, 24, and 48 h. In the next step, image analysis was carried out [49].

As a measure for the migration rate, the percentage removal of the lesional area was compared to the initial value using the following formula:

$$\text{scratch closure rate} = \left[\frac{(At_0 - Atx)}{At_0} \right] \times 100.$$

where At_0 = lesional area (cm^2) at time 0 and Atx = lesional area (cm^2) at time x .

Three independent tests were carried out for each cell type and each experimental setting, and mean values were calculated.

Digital Image Analysis

The photographed microscopic images were analyzed with respect to the size of the lesions over time. For this purpose, the margins of the cell layers were marked as measuring lines, and the lesional area was quantified with the calibrated number of pixels (ImageJ[®], V1.49m; National Institutes of Health, Bethesda, MD, USA). The values for these areas were compared with the corresponding initial value, and a scratch closure rate was calculated, serving as a measure for the cytological integrity of the respective cell type.

Biometric Evaluation

Biometric evaluation was carried out using the statistics software XLStat V2014.5.02 (Addinsoft, New York, NY, USA). The descriptive statistics are based on the results of the independent

individual experiments. The comparison of the independent random samples was carried out after the assessment of normal distribution and variance homogeneity using the one-way ANOVA model, the Student-Newman-Keuls method, the Holm-Bonferroni method, and the Kruskal-Wallis test. Differences between test and control results were assumed to be statistically significant at an error probability of $p < 0.05$.

Results

Penetration Tests

The concentration-time profiles from the diffusion tests with MMF on unaffected and damaged skin showed no relevant differences regarding the cutaneous bioavailability in the target compartments vital epidermis and dermis (Fig. 1). Nevertheless, a considerable reservoir was detected in the stratum corneum of intact skin. However, it had no pharmacokinetic relevance within the time frame up to 300 min. After 30 min, a relevant surplus in concentration could be found only in the vital epidermis of damaged skin, which was compensated after 300 min. Within the acceptor of both unaffected and damaged skin, the concentrations were below the detection limit. The mean concentration ranges of MMF within the target compartments vital epidermis (50–100 $\mu\text{mol/L}$) and corium (5–25 $\mu\text{mol/L}$) were used as a basis for the concentration range used in the cytotoxicological test (scratch assay).

Scratch Assay

The results from the scratch assay for cytotoxicology showed no concentration-depending or other relevant MMF-specific effects on keratinocytes or fibroblasts (Fig. 2). This also applies for the intrinsic effect of the solubilizing agent DMSO. However, regardless of the concentration, dispersion was greater for keratinocytes than for fibroblasts, which is especially apparent after 48 h. The level and time of the closure rate is comparable in both cell types.

Discussion

The present results for the pharmacokinetics of MMF correspond to the known characteristics of topically applied glucocorticoids [50]. The special feature here is the use of an O/W galenic preparation, which on the one hand suggests an improved release of the lipophilic MMF; on the other hand, however, no improved penetration into or permeation through the vital skin layers compared to an W/O preparation can be expected. This is as-

Fig. 1. Concentration profiles of mometasone furoate in comparison to intact and lesional skin and different application times (30, 100, and 300 min). Means and SD. SC, stratum corneum; EP, vital epidermis; DR, dermis; black bars, intact skin; gray bars, lesional skin.

sumed because the acceptor is very hydrophilic especially in acutely inflamed skin. Unfortunately, there are no pharmacokinetic data for a W/O preparation with the same active substance available, and, therefore, no direct comparisons regarding cutaneous bioavailability can be drawn. Clinical data, however, clearly show the therapeutic equivalence of both galenic systems [24]. Moreover, especially in inflamed skin, the use of an O/W vehicle is by all means advantageous from a clinical point of view (e.g. nonocclusive) as well as from the patients' perspective (e.g. nongreasy) [51]. The outer continuous aqueous phase causes evaporation chill, which has again a vasoconstrictive and therefore anti-inflammatory effect. Also, the occlusive intrinsic effect is considerably reduced com-

pared to a W/O preparation. However, with regard to the anti-inflammatory therapy of psoriasis, this effect can even be unfavorable especially when treating hyperkeratotic plaques. This is a known key argument for combined topical therapy of glucocorticoids with keratolytics (such as salicylic acid) for the initial treatment.

Comparing intact and lesional skin, the diffusion tests showed no relevant differences in pharmacokinetics especially regarding the cutaneous bioavailability in the target compartments vital epidermis and dermis. Although a considerable MMF reservoir in the stratum corneum of intact skin was observed, no influence on the diffusion kinetics into deeper layers could be determined within the investigated time frame of up to 300 min. On the one

Fig. 2. Photographic documentation (**a, b**) of dose-dependent scratch closure status at baseline, and 24 and 48 h after application of the test substance, and results of image analysis (**c, d**) of scratch closure rate at baseline, and 2, 4, 8, 12, 24, and 48 h after adding the test substance to keratinocyte (**a, c**) and fibroblast cultures (**b, d**).

hand, this corroborates the clinical data for therapeutic equivalence; on the other hand, no indications for a change in the safety profile are given. This includes both, the range of concentration-dependent adverse cutaneous effects as well as the potential systemic bioavailability [52]. The detectable concentrations of MMF in the vital epidermis (about 100 $\mu\text{mol/L}$) and in the dermis (about 20 $\mu\text{mol/L}$) were within a cutaneous toxicologically harmless dose range [21]. However, the current evidence for cutaneous toxicological effects is quite low, and whether the toxicological data from MMF inhalation can be directly transferred remains doubtful. Therefore, cytotoxicological data for keratinocytes and fibroblasts from the scratch test were collected. The results give no indication for relevant substance-specific effects. Thus, no relevant clinical symptoms caused by adverse cutaneous effects are to be expected, at least for short- and medium-term use. This was also proven by the currently available safety data from clinical studies [21, 24].

In conclusion, the use of an O/W galenic preparation for epicutaneous application of MMF is advantageous because of the intrinsic effects of the vehicle especially in periods of acute inflammation. Regarding intact and damaged skin, pharmacokinetic equivalence was proven.

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Statement of Ethics

All experiments were carried out in accordance with the guidelines of, and approved by, the ethics committee of the medical faculty of the Martin Luther University Halle-Wittenberg.

Disclosure Statement

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