

TOPICAL BIOAVAILABILITY OF GLUCOCORTICOSTEROIDS

Dermatopharmacokinetic and dermatopharmacodynamic
of topically applied triamcinolone acetonide
in humans

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Carolina Lucia Pellanda
aus Osogna (TI)

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Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät

auf Antrag von

Prof. Dr. phil. nat. Christian Surber

Prof. Dr. phil. nat. Georgios Imanidis

Prof. Dr. phil. nat. Hans Leuenberger

Basel, den 2. Mai 2006

Prof. Dr. sc. techn. Hans-Jacob Wirz
Dekan

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Abbreviations

AD	Atopic Dermatitis
ANDA	Abbreviated New Drug Application
ANOVA	ANalysis Of VAriance
CFR	Code of Federal Regulations
DHA	Dihydroxyacetone
DMAC	Dimethylacetamide
DMSO	Dimethyl sulfoxide
DPK	Dermatopharmacokinetic
EMA	European Agency for the Evaluation of Medicinal Products
FDA	Food and Drug Administration
HPLC	High Performance Liquid Chromatography
ICH	International Conference on the Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
MW	Molecular Weight
NDA	New Drug Application
NMF	Natural Moisturizing Factors
rpm	revolutions per minute
RSD	Relative Standard Deviation
SC	Stratum corneum
SCORAD	SCORing Atopic Dermatitis
SD	Standard Deviation $SD = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n - 1}}$
TACA	Triamcinolone acetonide
TEWL	Transepidermal Water Loss
TS	Tape Stripping
TTS	Transdermal Therapeutic System
UV	Ultraviolet
VIS	Visible

Summary

The aim of the present thesis was the *in vivo* investigation of the topical bioavailability of a model glucocorticosteroid, triamcinolone acetonide (TACA), using tape stripping. The layer by layer removal of the stratum corneum by tape stripping enables the quantification of drug amounts penetrated into the stratum corneum over time. This dermatopharmacokinetic (DPK) approach has been subject of fervent discussions in the past years, and concern about adequacy and reproducibility of the technique has been expressed. Yet, the successful performance of reliable and reproducible tape stripping investigations highly depends on the use of a standardized methodology and suitable analytical methods. This thesis proposed a standardized tape stripping protocol in combination with carefully validated analytical methods (Project I). After a proof of concept, the set of methods was applied in an *in vivo* investigation of the influence of different factors on topical bioavailability. Both pharmacokinetic and pharmacodynamic aspects ultimately determining the successful therapy outcome were investigated: the effect of dose and application frequency (Project II), the effect of occlusion (Project III), and the efficacy of a low-dose TACA formulation (Project IV). Concomitantly, the corticosteroid accumulation within the stratum corneum (reservoir development) was monitored, since a reservoir can considerably affect the therapy outcome and is particularly advantageous to prevent systemic side effects.

In **Project I**, the tape stripping technique was standardized and an HPLC method for TACA quantification on tapes after extraction was validated. The standardized tape stripping protocol included the use of a template (ensured the removal of stratum corneum samples from the same skin site) and a hand roller (ensured a constant pressure on the tape before stripping), and, most importantly, the removal of the entire stratum corneum of one skin site to cope with inter- and intra-individual differences in stratum corneum thickness. The HPLC method for TACA quantification was successfully validated and proved to have suitable specificity, linearity, accuracy, precision, and robustness in the working range. The combination of 1) standardized tape stripping as sampling method, 2) UV/VIS-spectroscopy for quantification of corneocytes (previously validated), and 3) the new validated HPLC method for quantification of TACA was then applied in a proof of concept with 6 healthy volunteers. TACA was applied on their forearm skin in either an acetonic solution or an ethanolic gel, and stratum corneum samples were removed by tape stripping after 0.5 h, 3 h, and 24 h. A clear vehicle effect on the TACA penetration could be observed. Whereas TACA deeply penetrated into the stratum corneum after application of the acetonic solution, the penetration after application of the ethanolic gel was only superficial (development of a skin surface reservoir). The method set proved to be suitable for the investigation of the TACA penetration into stratum corneum and was applied in a pharmacokinetic clinical trial with healthy volunteers (Projects II and III).

In **Project II**, the effect of dose and application frequency on the *in vivo* penetration of TACA into stratum corneum was investigated in 15 healthy volunteers. Dose and application frequency of topical corticosteroids are recurrently debated topics. Multiple-daily applications are common, although a superior efficacy compared to once-daily applications is not unequivocally proven. In the dose experiment, higher TACA amounts were quantified within the stratum corneum after application of a high dose ($300 \mu\text{g}/\text{cm}^2$ vs. $100 \mu\text{g}/\text{cm}^2$; acetonic solution). However, this difference was only significant immediately after application, and no difference was recorded at 4 h and 24 h. The application frequency experiment showed slightly higher TACA amounts within the stratum corneum after multiple application ($3 \times 100 \mu\text{g}/\text{cm}^2$) than after single application of the total dose ($1 \times 300 \mu\text{g}/\text{cm}^2$). As a result of multiple applications, the skin was periodically reloaded with new drug, thus achieving temporary higher amounts within the stratum corneum and redissolving potential TACA crystals. The still well quantifiable TACA amount retained within the stratum corneum at 24 h was rather due to the slow diffusion through the stratum corneum barrier than to a classical reservoir formation. The performance of a mass balance showed that a high TACA dose could result in faster stratum corneum permeation and higher systemic exposure, unwelcome in topical therapy. Thus, a low dose applied once daily may be preferable to higher doses.

In **Project III**, the effects of occlusion before (pre-occlusion) and after (post-occlusion) TACA application ($100 \mu\text{g}/\text{cm}^2$; acetonic solution) were investigated on the forearms of 10 healthy volunteers. Occlusion is known to enhance skin hydration and can induce the formation of a stratum corneum reservoir. Moreover, occlusion is clinically used to improve the efficacy of topical corticosteroids in severe forms of skin diseases. Pre-occlusion showed no effect on the TACA penetration into stratum corneum. In contrast, post-occlusion enhanced the TACA penetration by a factor of 2, favoring the development of a 24 h-lasting reservoir.

The efficacy of low-dose TACA in the treatment of atopic dermatitis was proved in **Project IV**, a double-blind, vehicle-controlled, randomized pharmacodynamic explorative study with half-side comparison in 14 patients. Low-dose TACA was added to a marketed skin care cream (Lichtena[®]) in a concentration which was 40 times lower than typical therapeutical corticosteroid concentrations (25 vs. $1000 \mu\text{g}/\text{g}$). Twice-daily application of the low-dose TACA formulation reduced the severity of the lesions (assessed by SCORAD) already after 1 week. In contrast, the cream base alone had no significant influence on the severity of atopic dermatitis measured for 1 month. These findings indicate that some corticosteroids may already be effective at much lower concentrations than usually used therapeutically, and that marketed corticosteroid formulations may contain a much higher concentration than necessary.

The investigations described in this thesis show how tape stripping, correctly performed, asserts itself as a valuable technique for topical bioavailability assessment. The DPK approach can be applied for the investigation of topical bioavailability of other compounds as well, provided that specific analytical methods for their quantification are developed and validated. Re-implementation of the DPK approach on regulatory level could be considered.

Aim of the thesis

The aim of this thesis was to investigate *in vivo* the topical bioavailability of a model glucocorticosteroid, triamcinolone acetonide (TACA), using tape stripping. The layer by layer removal of the stratum corneum by tape stripping enables the quantification of drug amounts penetrated into the stratum corneum over time. This DPK approach has been subject of fervent discussions in the past years, and concern about adequacy and reproducibility of the technique has led to the withdrawal of the corresponding FDA draft guidance “Topical dermatological drug products NDAs and ANDAs – *In vivo* bioavailability, bioequivalence, *in vitro* release, and associated studies” [1]. Currently, no technique is advised at regulatory level for the specific assessment of topical bioavailability.

Since the successful performance of reliable and reproducible tape stripping investigations highly depends on the techniques used, this thesis proposes a standardized tape stripping protocol in combination with carefully validated analytical methods (UV/VIS-spectroscopy, HPLC) to possibly rehabilitate the tape stripping technique (Project I). After proof of concept with 6 healthy volunteers, the set of methods was applied to investigate *in vivo* different factors which influence percutaneous penetration and which thus, ultimately, influence the successful outcome of a topical therapy. The therapeutical class of corticosteroids was chosen because it is still the gold standard for the therapy of several dermatological affections. Among the wide palette of corticosteroids, TACA as a commonly used and moderately potent steroid was chosen as model. Both pharmacokinetic and pharmacodynamic aspects were investigated.

The effect of the following pharmacokinetic parameters on the TACA penetration into stratum corneum was assessed in a clinical trial with 25 healthy volunteers: dose, application frequency, and occlusion. Dose and application frequency (Project II) of topical corticosteroids are recurrently debated topics. Multiple-daily applications are common, although a superior efficacy compared to once-daily applications is not unequivocally proven. Occlusion (Project III) is known to enhance the percutaneous penetration of many but not all drugs. Moreover, occlusion can induce the formation of a drug reservoir within the stratum corneum. A skin reservoir is desired in topical therapy, since the drug should remain for a long time at the site of action, exerting a local and not a systemic action. Finally, the efficacy of a low-dose TACA formulation was assessed in a pharmacodynamic clinical trial with 14 patients suffering from atopic dermatitis (Project IV).

This thesis is structured into a theoretical and an experimental section. The theoretical section gives an overview of: anatomy and physiology of the skin (Chapter 1); principles of percutaneous absorption and topical bioavailability (Chapter 2); techniques for the assessment of topical bioavailability and regulatory requirements (Chapter 3); skin reservoir (Chapter 4); and topical corticosteroids (Chapter 5). In this last chapter, both pharmacodynamic and pharmacokinetic aspects of dose, application frequency, and occlusion are reviewed. The experimental section describes the validation of the methodology (Project I) and the results of the *in vivo* investigations (Projects II-IV), submitted for publication in different scientific journals.