

Abstracts

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Wissenschaftliche Posterausstellung 2017: Poster 1

SmartLipids formulation for “natural” skin whitening

Hanna Aljarada (1), Sung Min Pyo (1), Rainer H. Müller (1), Cornelia Keck (2,3)

(1) Freie Universität Berlin, Institute of Pharmacy, 12169 Berlin, Germany

(2) Philipps Universität, Institute of Pharmacy, Robert-Koch-Str. 4, 35037 Marburg, Germany

(3) PharmaSol GmbH, Stubenrauchstr. 66, 12161 Berlin, Germany

SmartLipids, developed in 2014 [1], are the 3rd generation of lipid nanoparticles, after the first generation of solid lipid nanoparticles (SLN, 1991) and the nanostructured lipid carriers (NLC, 1999). This carrier system was used to develop a “base formulation” for more effective whitening formulations. The special delivery properties of SmartLipids are beneficial for delivery of whitening agents: homogenous film formation on skin, adhesive forces and thus prolonged residence time on the skin, occlusion effect by this film, and subsequently increased penetration of actives into the skin. Besides, the SmartLipids have skin caring effects, such as restoration of natural protective lipid film of the skin, and thus anti-pollution effect.

An increasing number of chemical synthetic whitening agents has run into problems of regulatory acceptance. Thus there is a trend towards natural compounds with whitening effect. This ranges from whitening oils (e.g. lemon oil, lemongrass oil, carrot seed oil) to plant extracts, e.g. licorice extract (glabridin as main active). SmartLipids are composed of a complex mixture of solid lipids with optionally liquid lipids (= oil). To have highest loading with whitening agents, it would be ideal to use an oil as part of the particle matrix mixture which has already whitening properties. At the same time this oil should have good dissolution properties for whitening agents, in this case glabridin.

Intensive screening of oils identified lemongrass oil having high solubility for licorice extract. Many oils dissolved only <1-3%, in lemongrass oil >25% dissolved. Based on this SmartLipid carriers were produced from a mixture of lipids containing e.g. beeswax, carnauba wax, Compritol 888 ATO, Miglyol 812 and lemongrass oil. A series of skin-friendly stabilizers was investigated regarding their ability to stabilize the particle suspensions, e.g. various Plantacare, alkyl polyglucosides, but also Tween. Most efficient were Plantacare 2000 UP and Tween 20. The Tween 20 formulation had a mean particle size of about 180 nm (photon correlation spectroscopy), laser diffraction diameters 50% of 150 nm and 99% of 370 nm. The particle sizes are above the critical 100 nm for nanoparticles, thus the “SmartLipids Whitening” are a submicron carrier. The measured zeta potential of -36 mV (in original dispersion medium) is a sufficiently high particle charge for predicting good physical stability.

With this a base formulation is available which has already on its own a whitening effect due to the lemon grass oil, and in which customer-specific whitening agents (e.g. glabridin) can be loaded.



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ml



Image analysis parameters to quantify the healing of superficial model wounds in vivo

Stephan Bielfeldt (1), Arne Böhling (1), Sabrina Laing (1), Klaus-P. Wilhelm (1)

(1) proDERM Institute for Applied Dermatological Research, 22869 Schenefeld, Germany

Introduction

A wide range of topical drugs and dermocosmetics are on the market that claims to improve the healing of superficial wounds. In vivo test methods on humans with minimal invasive abrasive model wounds that avoid formation of scars are available to proof such claims (2). For superficial wounds, different aspects of healing are of importance. Two important of them are: First, the early closure of the wound that stops the excessive transepidermal water loss (1, 3). And second, the active contraction of the wound that is caused by myofibroblast activity (4). In this poster we introduce image analysis methods for these two aspects of healing.

Material & Methods

Superficial wounds of approximately 10 mm in diameter were raised on the forearms of healthy subjects with the abrasive wound model (5). For 2 test products and untreated control the healing of the wounds was documented by highly reproducible advanced photography with a dermatoscope equipped with a CLR camera (Dermlite®, Canon EOS 5D, 24 M Pix). Photographs were taken directly after wound raising and on days 3, 5, 8, 9, 10, 12, 15. An image analysis tool was developed that allowed easy interactive color-marking of wound margins on the wound images. The experts who demarcate the wound margins could easily change the wound image size from original wound size up to 38 fold magnification without loss of image resolution. Processing of large sets of evaluated images by calculation of wound areas from the demarcation lines was performed automatically (5).

Results

The two parameters revealed distinctly different aspects of wound healing. Starting already on the first days of wound healing the formation of a thin layer spreading from the wound borders was observed. As a parameter for early wound closure the wound area that was not yet covered with a thin layer was demarcated. On the observed wounds it took about 12 to 15 days to close the wound with a thin layer. Distinct differences among the treatments were observed. The dimension of closure time fits well to previously published data where abrasive wounds treated with adhesive dressings were investigated (6).

To measure the contraction of the wound the original wound margin was demarcated. During the course of wound healing the wound area under all treatments decreased until day 9 and from then started to increase again, without coming back to original size even on investigation day 15. The wound contraction data reveals distinct treatment differences in onset, magnitude and



partially also degradation of myofibroblasts activity.

Conclusions

We conclude that the two presented imaging parameters are well applicable for abrasive wounds. Compared to direct clinical wound assessments they are more precise and objective and well verifiable by simple inspection of the demarcated images.

Literature

- (1) Czaika et. al., 2012, “Comparison of Transepidermal Water Loss and Laser Scanning Microscopy Measurements to Assess Their Value in the Characterization of Cutaneous Barrier Defects”, *Skin Pharmacol Physiol*, 25(1): 39–46
- (2) Wilhelm et. al., 2017, “Models of wound healing: an emphasis on clinical studies”, *Skin Res Technol*, 23(1): 3–12
- (3) Kottner et. al., 2013, “Characterisation of epidermal regeneration in vivo: a 60-day follow-up study”, *J wound care*, 22(8): 395–400
- (4) Desmoulière et.al., 2005, “Tissue repair, contraction, and the myofibroblast.”, *Wound Repair Regen*, 13(1): 7–12.



Wissenschaftliche Posterausstellung 2017: Poster 3

Measuring concentration depth profiles and stratum corneum thickness in vivo

Lisa Binder (a), Martin Wolf (a,b), Safoura Sheikh Rezaei (c), Andreas Baierl (d), Lukas Gruber (a), Michael Wolzt (c), Claudia Valenta (a,b)

(a) University of Vienna, Department of Pharmaceutical Technology and Biopharmaceutics, Althanstraße 14, 1090 Vienna, Austria

(b) University of Vienna, Research Platform 'Characterisation of Drug Delivery Systems on Skin and Investigation of Involved Mechanisms', Vienna, Austria

(c) Medical University of Vienna, Department of Clinical Pharmacology, Spitalgasse 23, 1090 Vienna, Austria

(d) University of Vienna, Department for Statistics and Operations Research, Oskar-Morgenstern-Platz 1, 1090 Vienna, Austria

Introduction

Confocal Raman spectroscopy (CRS) is an upcoming spectroscopic technique. It belongs to the field of vibrational spectroscopy and provides insight into the vibrational states of a molecule. The fact that it is non-invasive offers several advantages compared to other methods; an in vivo application is possible. In the area of skin research, this optical method allows the depth-resolved measurement into the skin, non-invasively and non-destructively.

The aim of the present study was to characterise the skin composition of the volar forearm using CRS. Skin components of interest were NMF and urea. These were selected according to their influence on the skin state and barrier function. Additionally, SC thickness was calculated from water concentration profiles.

Experimental methods

Study design

The study was conducted with approval from the Ethics Committee of the Medical University of Vienna. Fifteen randomly chosen Caucasian volunteers of both sexes, aged between 23 and 57 years, participated in the study. CRS experiments were carried out twice within 2 weeks.

During data evaluation, noticeable differences in CRS profiles were found that seemed to be linked to age. To assess this assumption more closely, two extreme age groups were defined: a young group consisting of two participants <25 years of age and an elderly group consisting of two participants >50 years of age.

Confocal Raman spectroscopy (CRS)

In vivo CRS experiments were carried out using a confocal Raman microspectrometer (gen2 Skin Composition Analyzer, River Diagnostics, Rotterdam, The Netherlands) with two incorporated lasers (671 nm and 785 nm). Fingerprint spectra were recorded from 0 up to a depth of 32 μm in 4 μm increments. Spectra in the high wavenumber region were obtained in 2 μm steps up to a depth of 40 μm . All spectra were measured on the volar forearm. At least five fingerprint profiles



and three high wavenumber profiles were collected and averaged for each volunteer. All spectra collected were analysed using SkinTools® software version 2.0, developed by River Diagnostics. The concentration of the skin components NMF, urea, ceramide and cholesterol was calculated as described by Caspers et al. [1]. Water profiles were generated by calculating the water content from the water to protein ratio. SC thickness was determined through water concentration profiles [2].

Results

The formation of NMF is reflected in the concentration profiles generated within the present study. NMF is formed by degradation of the protein filaggrin, which itself arises out of profilaggrin conversion. The transformation of filaggrin to NMF starts at the lowermost part of the SC and proceeds through the upward layers. In deeper skin areas, filaggrin does not persist. Our findings showed a substantially increased NMF concentration at the skin surface compared to deeper layers.

Age-dependent variations of the skin were examined. Thereby, an elevated amount of NMF in aged skin was found.

In contrast to NMF, the urea concentration profiles did not show a consistent progression. For one group of the volunteers, within 32 µm depth almost no changes in the urea concentration were found. The other group showed a considerable higher urea concentration at the skin surface, followed by a rapid drop. The comparison of the two did not reveal any significant age-related differences.

CRS-derived SC thicknesses varied between 12 and 26 µm. SC thickness was expected to increase with age; indeed, notable differences between the age-group of under-25-years-old and the participants over 50 years of age were found. The apparent SC thickening with age may be caused by the also observed decline in the water concentration.

In conclusion, depth profiles showed a comparable curve progression for the tested individuals and age-related differences in skin composition were found. CRS serves as useful tool to investigate skin properties fast and without the need for pre-treatment.

Acknowledgements

The financial support of the research platform “Characterisation of Drug Delivery Systems on Skin and Investigation of Involved Mechanisms” is gratefully acknowledged.

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Wissenschaftliche Posterausstellung 2017: Poster 4

Modification of the Dermal Barrier Function by Atmospheric Pressure Plasma

M. Gelker (1, 2), C. Mueller-Goymann (2, 3), W. Vioel (1)

(1) University of Applied Sciences and Arts, Faculty of Natural Sciences and Technology, Von-OssietzkyStr. 100, 37085 Goettingen, Germany

(2) PVZ – Center of Pharmaceutical Engineering, Technische Universität Braunschweig, Franz-Liszt-Str. 35a, 38106 Braunschweig, Germany

(3) Institut Pharmazeutische Technologie, Technische Universität Braunschweig, Mendelssohnstrasse 1, 38106 Braunschweig, Germany

The outer layer of mammalian skin, the stratum corneum (SC), acts as the main barrier to protect the body from environmental influences and potentially hazardous substances. However, the skin is a popular route for drug delivery and especially newly developed drugs, which are often large and poorly water-soluble molecules, may benefit from dermal delivery.

The goal of our research is to enable cutaneous drug delivery by an efficient, safe and pain-free method using cold atmospheric plasma (CAP). It has recently been shown that CAP can be employed to enhance skin penetration of various substances [1, 2]. The beneficial properties of CAP sources similar to those used in our experiments have already been used in wound care [3]. Therefore, future CAP treatments, e.g., of skin disorders, may distinctly benefit from synergistic effects.

In our work, plasma-assisted permeabilization of model systems (e.g., isolated human SC) by custom-made CAP sources is tested using easily detectable model pharmaceuticals differing in size from below 1 nm to 500 nm. Electrical resistance measurements and the results of Franz diffusion cell studies with isolated human SC show an alteration of the barrier function as to reduced electrical resistance values and enhanced permeation of fluorescent marker molecules which is dependent on the treatment duration. While a single treatment for 90 s at $212 \pm 20 \text{ mW} \cdot \text{cm}^{-2}$ did significantly reduce SC electrical resistance, it did not significantly alter permeation of fluorescent model drugs as compared to control samples. Two 90 s CAP-treatments did both decrease the electrical resistance and significantly facilitate permeation of marker molecules with a molecular weight of up to 500 kDa (Stokes radius $\sim 14,7 \text{ nm}$).

These results will on the one hand contribute to the safety assessment of CAP-sources used in medical care but also offer further insight into the innovative field of CAP-assisted drug delivery.

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Wissenschaftliche Posterausstellung 2017: Poster 5

Fibroblasts from Aged Donors Shape the Morphology of Reconstructed Human Skin Towards an Aged Skin Phenotyp

Christian Hausmann (1), Roberta Balansin Rigon (1, 2), Barbara Wanjiku (1), Christopher Wolff (1), Zijun Deng (3), Maxim Darvin (4), Fabian Schumacher (5), Michaela Sochorova (6), Andrej Kovacik (6), Julia Tigges (7), Jürgen Lademann (4), Ellen Fritsche (7), Burkhard Kleuser (5), Kateřina Vávrová (6), Nan Ma (3), Christian Zoschke (1), Monika Schäfer-Korting (1)*

(1) Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany

(2) School of Pharmaceutical Sciences, São Paulo State University, UNESP, São Paulo, Brazil

(3) Institute of Biomaterial Science, Helmholtz-Zentrum Geesthacht, Teltow, Germany

(4) Charité – University Medicine Berlin, Berlin, Germany

(5) Institute of Nutritional Science, Universität Potsdam, Potsdam, Germany

(6) Faculty of Pharmacy, Charles University Prague, Hradec Kralove, Czech Republic

(7) IUF - Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany

* Corresponding Author:

Prof. Dr. Monika Schäfer-Korting Freie Universität Berlin, Institute of Pharmacy (Pharmacology and Toxicology) Königin-Luise-Str. 2+4, 14195 Berlin, Germany Phone +49 30 838-53283; Fax+49 30 838-470871 E-mail monika.schaefer-korting@fu-berlin.de

Increasing numbers of multimorbid, elderly patients challenge translational pharmacology, which predominantly relies on young and healthy test subjects and animal models [1]. Several groups tried to model aged human skin in vitro, facing specific restrictions [2]. We studied the effects of normal human dermal fibroblasts (NHDF) from donors with varying age and sex on the morphology of reconstructed human skin (RHS). NHDF were either isolated from fore-skin (medically-indicated circumcision of <9 year-old boys; juvenile RHS) or from breast skin (plastic surgery; 60 to 70 year-old women; aged RHS). Keratinocytes from the juvenile donors (<9 years) were used for the epidermal compartment of all RHS to investigate the influence of NHDF age and origin on epidermal development.

Microarray analysis revealed decreased collagen-1 and -3 expression only in RHS, but not in monolayer cultures. Sirtuin-1 and mitochondrial transcription factor-1 as well as apoptosis-related gene expression of E1A binding protein p300 declined with donor age. Dermal thickness, collagen content, and fibroblast count decreased markedly in aged RHS, whereas matrixmetalloproteinase-1 gene and protein expression increased. This is well in accordance to in vivo studies [3-6]. A thinner viable epidermis at the expense of a thickened stratum corneum and a decreased surface pH were observed in aged RHS, again being well in line to aged skin physiology [7]. Decreased free fatty acid content in the stratum corneum of aged RHS and increased amounts of cholesterol, cholesterol sulfate, and ceramide, in particular increased sphingosine-



and dihydrosphingosine based ceramides, indicate an overall increase in barrier lipids. This might explain the slight decrease of caffeine permeation as well as the faster penetration of tacrolimus into aged RHS, given the very poorly penetrating high molecular and very lipophilic drug is entrapped in the stratum corneum. Taken together, fibroblasts do not only shape the dermal compartment, but also affect the epidermal differentiation and thus barrier function. Understanding age-related changes in the barrier function will allow improving the dermatological treatment for the increasing number of aged patients – and might reduce animal testing as well.

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Wissenschaftliche Posterausstellung 2017: Poster 6

Film-Forming Formulations with Sustained Penetration of an Antipruritic Drug into the Skin

Rouven Heck (1), Milica Ž. Lukić (2), Snežana D. Savić (2), Rolf Daniels (1) and Dominique J. Lunter (1)

(1) Department of Pharmaceutical Technology, Eberhard Karls University, Tuebingen, Germany

(2) Department of Pharmaceutical Technology and Cosmetology, Faculty of Pharmacy, University of Belgrade, Serbia

Chronic pruritus is a common symptom accompanying various chronic skin diseases. Conventionally, it is treated with antihistamines and local anesthetics. However, these drugs often cannot provide sufficient relief. As an alternative, capsaicinoids can be used. Their long-lasting antipruritic effect is caused by continuous stimulation of TRPV1 at the epidermal pain conducting fibers. To achieve this, currently available formulations need to be applied 4-6 times a day. This is inconvenient and results in poor patient compliance.

The aim of our study was to develop a film-forming formulation (FFF) with sustained release for dermal use making it easy to treat large areas of affected skin over a long period. Nonivamide (synthetic capsaicin) was used as active.

FFFs were prepared by loading a solution of nonivamide in refined castor oil into mesoporous silica. This was subsequently incorporated into a plasticized film-forming polymer dispersion. Film forming capacity of the FFFs was investigated by confocal Raman microscopy. Color coded images show that the oil is bound to the silica and immobilized in a polymeric matrix. The inclusion of the nonivamide containing oil was regarded as a prerequisite to achieve sustained penetration into the skin.

Ex vivo permeation experiments were carried out to parametrically compare permeation of nonivamide from FFFs to a standard formulation (Hydrophilic Nonivamide Cream; HNC; prepared according to "Hydrophile Capsaicinoid Creme" in: Neues Rezeptur Formularium; monograph #11.125). It was found that permeation rate from a FFF with 0.9 % nonivamide was comparable to that from HNC containing 0.05 % nonivamide. The permeation rate from the FFF falls thus into a therapeutically suitable range.

As the site of action of capsaicinoids is located within the viable epidermis, ex vivo penetration experiments were performed to compare nonivamide penetration from FFF and HNC into excised skin. It was found that the FFF was capable of delivering a similar amount of nonivamide to the skin as the HNC. Nonivamide levels in the viable epidermis decreased rapidly if it was applied in HNC but were kept constant over a period of 24 hours if it penetrated from FFF. The capability of FFF to sustain penetration was thus shown.



Furthermore, skin irritation potential of FFF was tested against the vehicle and the control formulation HNC in an in vivo experiment in human volunteers (signed written consent obtained, approved by local ethics committee and in accordance with the declaration of Helsinki). Transepidermal water loss, skin hydration and erythema index were assessed. It was found that FFF did not alter any of the measured parameters within the application time. This shows the excellent skin tolerability of the FFF.

Our investigation clearly shows that FFFs exhibit the desired sustained penetration profile while being well tolerated. As a result, dosing intervals can be prolonged and patient compliance to the treatment can be improved.



A novel strategy to assess drug delivery kinetics to epidermal targets in vivo

Magdalena Hoppel (1), M. Begoña Delgado-Charro (1), Richard H. Guy (1)

(1) University of Bath, Department of Pharmacy & Pharmacology, Bath, UK

Introduction:

It has proven difficult to quantify 'drug input' from a formulation to the viable skin because the epidermal and dermal targets of topically applied drugs are hard, if not impossible, to access in vivo. Defining the drug input function from a formulation to the viable skin with a straightforward and practical experimental approach would enable a key component of dermal pharmacokinetics to be characterised. Stratum corneum (SC) tape-stripping has been used to measure drug uptake from a formulation after a defined period of application; by delaying tape-stripping post-removal of the formulation, it is also possible to assess drug clearance from the SC. It is hypothesised that the difference between uptake and clearance measurements allows estimation of a topical drug's input function into the viable tissue [1]. This study aimed to test this idea by comparing the input of lidocaine into the viable skin, following application of commercialised patch and cream products, using SC tape-stripping in vivo with that determined more conventionally in vitro.

Methods:

Twelve healthy human volunteers participated in the in vivo SC tape-stripping study, which was approved by the Research Ethics Approval Committee for Health of the University of Bath. On separate occasions, either a Versatis® 5 mg medicated plaster or LMX4 cream (lidocaine 4% w/w) was applied to both forearms. Drug uptake into, and clearance from, the SC were measured immediately following 12 hr of patch application, and 4 hr and 8 hr post-patch removal, respectively; for the cream, the uptake time was 1 hr, the clearance times were the same as those for the patch. In vitro experiments used dermatomed, abdominal pig skin (750 µm) and Franz diffusion cells ($n \geq 6$ for each formulation); the receptor solution was PBS buffered at pH 7.4.

Results and discussion:

The in vivo SC uptake and clearance data provided estimates of the lidocaine input rate into the viable skin tissue of $11.5 \pm 2.3 \mu\text{g cm}^{-2} \text{h}^{-1}$ and $5.3 \pm 2.8 \mu\text{g cm}^{-2} \text{h}^{-1}$ from the cream and patch, respectively. The significantly higher delivery of the drug from the cream compared to the patch was confirmed qualitatively and quantitatively in vitro. From the estimated steady state flux of lidocaine from the patch in vivo, and the amount of drug cleared from the SC post-removal of the formulation, the total delivery was determined to be $\sim 110 \mu\text{g cm}^{-2}$. This value agrees very well with the claimed [2] lidocaine absorption of $150 \pm 100 \mu\text{g cm}^{-2}$. In conclusion, the results support the hypothesis that drug input into the viable skin from a topical formulation can be estimated using SC tape-stripping at judiciously selected uptake and clearance times.



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Acknowledgements

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Wissenschaftliche Posterausstellung 2017: Poster 8

“BergaCare SmartLipids Retinol” – product features for improved dermal retinol delivery

Florence Olechowski (1), Sung Min Pyo (2), Cornelia Keck (3), Rainer H. Müller (2)

(1) Berg + Schmidt GmbH & Co. KG, 20099 Hamburg, Germany

(2) Freie Universität Berlin, Institute of Pharmacy, 12169 Berlin, Germany

(3) Philipps Universität, Institute of Pharmacy, Robert-Koch-Str. 4, 35037 Marburg, Germany

The BergaCare SmartLipids are a novel dermal delivery system developed in 2014 [1], the successor generation after the solid lipid nanoparticles (SLN) and (NLC). They combine the advantages of SLN and NLC with new features, mainly increased loading capacity, higher physical stability and firm inclusion of the active during storage (no expulsion of cosmetic/pharma active from carrier due to polymorphic transition of the particle matrix lipids). BergaCare SmartLipids are able to protect chemically labile actives against degradation, thus they are a highly suitable carrier system for formulating the labile retinol. The problems of dermal retinol products are: a) chemical instability, b) suboptimal release to the skin (too high initial concentrations), c) suboptimal penetration profile from emulsions and d) potential skin irritation at effective retinol concentrations due to suboptimal release.

The solution for these problems is the formulation of retinol into BergaCare SmartLipids particles. The BergaCare SmartLipids proved to effectively stabilize retinol. Storage at room temperature: after 6 months still 94% retinol remained in BergaCare SmartLipids, whereas already after 4 months only 78% remained in the reference emulsion. When storing the retinol BergaCare SmartLipids in the fridge, 100% retinol were recovered after 6 months.

In comparison to emulsions, the BergaCare SmartLipids show a prolonged in vitro release (40%), versus undesired burst from the reference retinol emulsion (80%) (USP paddle method). Skin penetration was studied using pig ear skin and the tape stripping test. Relative high concentrations of retinol were found in the upper cell layers when released from the emulsion. In contrast, retinol released from lipid particles avoided such high concentrations, substantial retinol amounts were found in deeper layers.

The modified release pattern is the basis to avoid skin irritation and redness. Lipid particles loaded with retinol were used in an anti-couperosis formulation and tested in a human case study. Improvement was clearly seen within 5 days after laser treatment, and long-term treatment (> 1 year) avoided the often happening re-occurrence of the couperosis.

The particle concentration of the BergaCare SmartLipids concentrate is 17%, retinol content 2.3%. The concentrate can simply be admixed to the water phase of dermal products directly after their production (e.g. in cooling down phase), dilution factor is about 20 (depending on



the desired retinol concentration in the final market product).

References:

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Wissenschaftliche Posterausstellung 2017: Poster 9

Treatment of chronic urticaria in children – A cross-sectional analysis of specialized dermatological care in pediatric patients

Adriane Peveling-Oberhag, Sebastian Zimmer, Berenice Lang, Dennis Maeck, Anja Weber, Tom Gilfert, Petra Staubach

Department of Dermatology, University Medical Center of Mainz, Johannes Gutenberg-University Mainz, Mainz, Germany

Background:

Chronic urticaria (CU) is a common disease occurring in all ages. But CU in children has been devoted less attention so far. International management guidelines for pharmacotherapy in children derive largely from evidence in the adult.

Objective: To examine the clinical presentation, disease burden and pharmacological treatments in childhood to develop strategies for effective management of children with CU.

Methods:

200 children (0-17 years, 57% females) with CU were included in a standardized extended diagnostic program in the specialized urticaria outpatient clinic, Department of Dermatology, University Medical Center Mainz, Germany from 2012-2015.

Results:

The disease duration at time of presentation ranged from 2 months to 9 years. 62.5% presented with chronic spontaneous urticaria (CsU), 28% with chronic inducible urticaria, and 9.5% showed a combination. 15% of patients had not received any treatment. The majority (73%) were treated with second generation antihistamines (single dose) as monotherapy. Only 4 patients received an updosing (doubled recommended daily dose) as suggested by guidelines. 53% showed persisting symptoms despite therapy. Of these insufficiently treated patients 60% received single-dose second generation antihistamines without updosing. All eight patients (4%) on first generation antihistamines reported ongoing symptoms and also all patients with additional montelukast treatment did not have full symptom control. Eleven patients (8.3%) received steroid pulse therapy in addition to antihistamines with consecutive symptom control in all cases.

Conclusion:

The current data suggest a significant pharmacological undertreatment in children with CU. Although more than half of the patients were symptomatic under therapy with single-dose second generation antihistamines, no updosing or change of medication was performed. Treating physicians should be alerted to existing options of treatment escalation in pediatric CU and further investigations are urgently necessary to optimize the management of CU in children.



Wissenschaftliche Posterausstellung 2017: Poster 10

Micellar and non-micellar transport with various membranes

Julia Puschmann (1, 2), Michael Herbig (2), Christel C. Müller-Goymann (1)

(1) Institut für Pharmazeutische Technologie, TU Braunschweig

(2) Almirall Hermal GmbH

Introduction:

The partition behaviour of various components of topical formulations within the different phases (oil, micelles, aqueous) is an important aspect. Distribution of API might have high impact on the stability regarding possible degradation mechanism in the aqueous phase. The Dianorm® Equilibrium Dialyser with its two-chamber-system shall be used for the determination of the micellar and free transport through membranes with various molecular weight cut offs (MWCO) to measure the amount of API in the aqueous phase. Whether micellar solubilisation in water prevents or supports API degradation has to be investigated [1,2]. Furthermore a non-micellar solubilised substance is used as a control substance to guarantee the permeability of the membranes. Polysorbate 80 (PS) in different concentrations was chosen as a surfactant with known CMC and micellar molecular weight to differentiate between micellar and free transport [3].

Methods:

Various PS mixtures (0.15%, 1.5%, 5.0% (w/w)) in citric buffer pH 5 were prepared. Donor media contained betamethasone dipropionate (BDP) and phenoxyethanol in the PS mixtures. Additionally, emulsion gels containing 0.5% phenoxyethanol with varying PS-concentrations were tested. Donor and acceptor medium (citric buffer) were filled in PTFE-cells (n=5) separated by membranes and equilibrated at 25 °C for 24 h. Membranes made of hydrophilic cellulose ester (HCE) with various MWCO (0.5 - 100 kDa) were used and extracted in methanol. Samples were analysed via UPLC with PDA detector. Effective permeability coefficient (Peff) was calculated for BDP and phenoxyethanol [4]. An equivalence test was performed to control the equilibrium of donor and acceptor media ($\alpha=0.05$).

Results:

BDP-solubility increased linearly with increasing PS-concentration due to micellar solubilisation. The impact of the MWCO on the substance transport was measured with the 1.5% PS mixture. 0.5 kDa HCE membrane inhibited BDP transport as the molecular weight of BDP is 505 Da [5]. With increasing MWCO more BDP diffused into the acceptor phase. Only the 100 kDa membrane allowed equilibrium due to the enabled micellar transport. However, the recovery rate decreased (0.5 kDa: 89.5%, 100 kDa: 53.9%). It is assumed that BDP undergoes binding reactions with the membrane.

Three different PS mixtures comparing the 5 kDa and 100 kDa membranes were dialysed. Equilibrium for phenoxyethanol was reached within 0.15% and 1.5% PS mixtures ($p<0.05$).



The PS-concentration and the varying MWCO of the membranes had no effect on the P_{eff} of phenoxyethanol ($P_{eff}=0.6\pm 0.07\times 10^2$ cm/h). The P_{eff} of phenoxyethanol of dialysed emulsion gels was $0.3\pm 0.02\times 10^2$ cm/h ($n=44$). The reduction of P_{eff} between PS mixtures and emulsion gels is caused by the additional partition of phenoxyethanol in the oil phase of the emulsion. Around 4.3 times lower P_{eff} -values of BDP were detected with increased PS-concentration. Reason for this is the enhanced micellar solubilisation of BDP. Due to enabled transport of micelles P_{eff} increased with higher MWCO (5 kDa: $P_{eff}=1.9\pm 0.17\times 10^2$ cm/h; 100 kDa: $P_{eff}=2.6\pm 0.09\times 10^2$ cm/h).

Conclusion:

Phenoxyethanol was detected as an indicator for the effective membrane permeability. As a non-micellar solubilised substance it supports the differentiation between free and micellar drug transport. The effect of varying oil and emulsifier systems on the partition behaviour regarding the degradation of BDP and the antimicrobial effect of phenoxyethanol has to be determined.

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Electron Microscopy and Reflectance Confocal Microscopy Reveal Altered Epidermal Differentiation following Collagen Glycations

*Roberta Balansin Rigon (1, 2), Sabine Käbmeyer (3), Christian Hausmann (1), Marlus Chorilli (2), Christian Zoschke (1), Martina Ulrich (4), Monika Schäfer-Korting (1)**

(1) Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany

(2) School of Pharmaceutical Sciences, São Paulo State University, UNESP, São Paulo, Brazil

(3) Institute of Veterinary Anatomy, Freie Universität Berlin, Berlin, Germany

(4) Collegium Medicum Berlin, Berlin, Germany

** Corresponding Author: Prof. Dr. Monika Schäfer-Korting*

*Freie Universität Berlin, Institute of Pharmacy (Pharmacology and Toxicology)
Königin-Luise-Str. 2+4, 14195 Berlin, Germany*

Glycation of proteins such as collagen impairs their functionality and triggers pro-inflammatory pathways as well as increases oxidative stress within the tissue [1]. Since advanced glycation end products (AGEs) accumulate with donor age, glycation matters not only in diabetes patients, but also presents a hallmark of intrinsic skin aging. Previous studies found decreased filaggrin and loricrin expression, suggesting an impaired skin barrier function.

Herein, we studied the effect of collagen glycation on the epidermal morphology and the barrier function of reconstructed human skin (RHS). Normal human dermal fibroblasts and keratinocytes were isolated from juvenile foreskin. Collagen was pre-incubated with ribose to induce AGE products formation. Fluorescence spectroscopy confirmed collagen glycation. The effects of glycated collagen were compared to RHS with non-glycated collagen. Glycated and non-glycated RHS were subjected either to electron microscopy or to reflectance confocal microscopy.

Reflectance confocal microscopy swiftly analyzes RHS morphology without damaging the constructs. In accordance with in vivo data, collagen appeared more lumpy in glycated than in non-glycated RHS. Moreover, reflectance confocal microscopy revealed increased epidermal thickness of glycated RHS. Electron microscopy confirmed a thicker stratum corneum with plenty of cell layers of stratum granulosum at the expense of the stratum spinosum of glycated RHS. Moreover, the sharp distinction between stratum corneum and granulosum vanishes. Keratohyalin granula appear fine-grained and distributed throughout the keratinocytes within glycated RHS compared to non-glycated RHS, where the cells reveal more aggregated granules. The altered morphology of keratohyalin granula points to differences in keratinocyte differentiation [2]. No differences were observed in keratinocytes in the stratum basale: both glycated and non-glycated RHS are closely attached to the dermal compartment by an intact



basal lamina. Differences in collagen structure become also visible by electron microscopy. Caffeine permeation decreases by about 15% compared to non-glycated RHS, being well in accordance to in vivo studies with intrinsically-aged volunteers [3].

In conclusion, both electron microscopy and reflectance confocal microscopy prove a thicker stratum corneum with hallmarks of altered epidermal differentiation of reconstructed human skin with glycated collagen. Further studies will focus on the molecular cross-talk between glycated collagen and epidermal differentiation.

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The bitter taste receptor agonists amarogentin and Gentiana lutea extract modulate cell differentiation and lipid synthesis in keratinocytes

Ute Wölfle (1), Birgit Haarhaus (1), Floriana A. Elsholz (2), Walter E. Müller (2), Christoph M. Schempp (1)

(1) Research Center skinital, Department of Dermatology, University Medical Center Freiburg
(2) Department of Pharmacology, Biocenter, Goethe University, Frankfurt, Germany

We have recently shown that human keratinocytes express the human bitter taste receptors TAS2R1 and TAS2R38. Here, we have analyzed if TAS2R agonists induce the synthesis of skin barrier proteins and lipids in keratinocytes.

HaCaT keratinocytes were stimulated with the TAS2R agonist amarogentin, a characteristic bitter agent of Gentiana lutea extract (GE). Amarogentin induced calcium influx and promoted the expression of keratin 10, involucrin and transglutaminase.

Furthermore we analyzed if GE additionally has an effect on lipid synthesis in keratinocytes. To address this issue, we performed a quantitative fluorescence assay with the dye Nile Red that is only fluorescent in a hydrophobic environment. Primary keratinocytes were incubated for 6 days with GE. Nile Red labeling revealed that GE significantly increased lipid synthesis in keratinocytes. No toxic effects of amarogentin and GE could be detected.

Because epidermal proteins and lipids are essential for building an intact epidermal barrier, amarogentin and GE might be used to improve skin conditions with an impaired barrier function.

