Abstracts

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Wissenschaftliche Posterausstellung: Poster 1

Transcutaneous route for targeting antigen presenting cells by HIV-1 virus-like particles

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The idea of easy accessibility of cutaneous antigen presenting cells (APC) fostered the development of various transcutaneous (t.c.) vaccination strategies. Binding of vaccine to particles could improve skin penetration and APC activation. Virus-like particles (VLPs) are highly interesting carrier structures because they are biodegradable and because candidate antigens are incorporated in the particle's capsid.

We investigated the effect of skin vaccination strategies on the cellular uptake and penetration of three different VLP models, carrying HIV-1 antigen, on the skin tissue explants. Non-invasive t.c. administration after cyanoacrylate tape stripping (CSSS), was compared to skin pricking, and intradermal (i.d.) injection. To evaluate the migration of activated APCs out of the skin tissue, we established a skin culture model for human skin explants. The isolated epidermal and dermal cells as well as migrated cells from the medium were stained with HLA-DR marker and analysed by flow cytometry and fluorescent microscopy. Skin penetration of fluorescently labelled VLPs was assessed microscopically on cryosections.

In almost all skin samples, we were able to isolate VLP-positive cells from epidermal and dermal cell suspensions. Interestingly, the highest uptake was observed with VLP-Pr55gag, which could be a result of faster migration activity in response to this special VLP-type.
Although cellular VLP uptake in epidermis and the dermis was higher in pricked skin, the number of VLP-positive migratory cells after 40 hours did not significantly differ from CSSS-treated skin. In case of i.d., uptake was mainly in dermal and not in epidermal APCs.

These results suggest that the non-invasive method could have the same efficacy in the activation and migration of APCs and eventually the immune response afterward. The differences observed between the different VLPs on APC migration rate could be due to different antigenic properties of each VLP vaccine model.

Wissenschaftliche Posterausstellung: Poster 2

Cell response of the human keratinocytes cell line HaCaT to differently produced silver nanoparticles

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New antimicrobial agents based on silver are investigated and improve the treatment of topical bacterial infections. Silver nanoparticles (AgNP) represent a promising achievement of nanotechnology in health care and medicine. Over the last years, AgNP have been employed in several products as for example in antimicrobial gels and wound dressings. First investigations of AgNP showed an increased antibacterial potential caused by a slower but continuous release of silver ions (Ag+). On the other hand, toxic effects due to the AgNP themselves cannot be excluded. However, it has been shown that silver can affect bacterial as well as eukaryotic cells. It could increase the reactive oxygen species (ROS) production which leads to oxidative stress.

The present study investigates influences of AgNP on a human keratinocyte cell line (HaCaT) by means of transmission electron microscopy (TEM), a cell viability assay (XTT) and electron paramagnetic resonance (EPR) spectroscopy. The release of Ag+ ions from AgNP is due to an oxidative reaction on the particle surface and it can be reduced by producing and storing particles under inert atmosphere, e.g. argon (Ar).Thus, to distinguish between particle and ion induced effects, two systems were investigated. One batch of AgNP produced and stored under air (O2) was compared to particles produced under Ar. We have shown the intracellular uptake of AgNP by HaCaT cells using TEM. It could be demonstrated that the particles accumulated in vesicles and did not enter the nuclei. The incubation with silver led to morphological changes of the nuclei and the cell membrane, which indicates cell death. These results could also be confirmed by the XTT assay. We found a silver concentration dependent decrease in the cell viability. To find out if the detected toxicity relates to an increased ROS production, intracellular radicals were measured. Higher ROS levels were detected in cells incubated with AgNP. Moreover, it was also possible to measure significant differences between the two particle types. To our knowledge, EPR spectroscopy was used for the first time to show ROS production induced by AgNP in HaCaT cells.

In conclusion, AgNP made and stored under air induced higher amounts of ROS compared to

the AgNP stored under Ar. This suggests that Ag+ ions released during particle storage are responsible for most of AgNP related cytotoxic effects. Nevertheless, the reduction in cell activity found for AgNP (Ar) suggests that effects caused by the nanoparticles themselves and ions released after particle uptake might also exist. The results highlight the complexity of silver toxicity towards skin cells, which need to be thoroughly evaluated for safe use of these nanomaterials in dermatology and other medical areas.

Wissenschaftliche Posterausstellung: Poster 3

Interaction between human skin and sunscreen-loaded nanosuspensions from beeswax and jojoba oil: a DSC-study

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Nanosuspensions composed of titanium dioxide as inorganic sunscreen within a matrix of beeswax and jojoba oil in a 2:1 ratio and stabilized by eudermic surfactants were previously investigated relating to their sun protection and particle size distribution [1]. In this study the focus will be on the interaction of stratum corneum (SC) with these nanosuspensions and the respective surfactant solutions.

Differential scanning calorimetry (DSC) enables the detection of changes in SC lipid arrangement affected by the interactions with formulation ingredients. Four endothermic transitions T1–T4 are observed by means of DSC when SC is heated up to 120 °C [2-5]. While T1 (~40 °C) and T4 (~100 °C) are not always visible, the presence of T2 (~70 °C) and T3 (~85 °C) are more reliable. T1 and T4 were found to be strongly dependent on the skin source and its water content during measurement [4]. According to Leopold and Lippold, shifts lower than 3.0 °C are considered statistically significant [5].

Methods: Nanosuspensions were manufactured by dispersing a molten lipid phase consisting of beeswax and jojoba oil at the ratio of 2:1 into an aqueous phase by using high-pressure homogenization. Either sodium lauroyl sarcosinate (SLS), sucrose laurate (SL) or potassium stearate (PS) were used as surfactants in the aqueous phase.

For DSC experiments, hydrated SC with a water content of 20 % was incubated at 37 °C for 30 minutes with the suspensions and surfactant solutions, respectively. Samples were sealed in aluminium pans and subsequently measured from 20 °C to 120 °C with a heating rate of 5 K/min and an empty aluminium pan as reference using DSC 1 Stare System with HSS7 sensor (Mettler Toledo, Schwerzenbach, Switzerland). Furthermore suspensions and surfactant solutions were measured without SC from 20 °C to 105 °C, cooled down to 5 °C and reheated up to 105 °C with the same heating rate of 5 K/min.

Up to four endothermic thermal transitions were recorded, indicated as T1–T4, but only the T3 and T4 were reliable for further interpretation. The transition temperature evaluation was made using software STARe V10.00.

Results: Nanosuspensions showed a broad melting event around 60 °C (peak maximum). Due to the combination of solid beeswax and liquid jojoba oil as the lipid matrix there is no sharp melting peak, but a broad melting range. Upon cooling recrystallization occurred around 52 °C. With increasing amount of SLS (1 % up to 5 %) a slight increase in onset temperature was

observed. A similar phenomenon resulted from formulations containing SL as surfactant. In contrast to this, nanosuspensions with PS as surfactant exhibited an exothermic event around 90 °C, possibly because of evaporation and/or decomposition. With increasing concentration of PS the onset temperature of the exothermic event shifted slightly to lower temperatures.
After incubation of the SC with different nanosuspensions it was noticeable that the broad melting peak resulting from the lipid matrix coincided with the endothermic transition shift T2, therefore the emphasis was placed on the endothermic transition shift T3. In comparison to untreated SC (T3: 86.87 ± 1.10 °C) and with increasing surfactant concentrations the endothermic transition shifts became more visible (see Table 1). As an example, T3 shifted for about -10 K formulations of 10 % of PS or SL, respectively. Low concentrations of 1 % surfactant showed a minor change of T3 by about -6-8 K.

Table 1: Endothermic transitions T2-T4 after incubating the SC with nanosuspension or surfactant solution

	Nanosuspension * T3 [°C]	T4 [°C]	Surfactant solution Δ T2 [°C]	T3 [°C]	T4 [°C
SLS					
1%	79.86 ± 0.77	93.22 ± 0.89		71.99 ± 0.89	90.82 ± 0.64
2 %	79.56 ± 0.10	93.89 ± 0.45		71.53 ± 0.44	89.15 ± 0.36
5 %	77.15 ± 0.56	91.72 ± 0.27		72.32 ± 1.22	88.07 ± 0.78
SL					
1 %	78.42 ± 1.05		68.77 ± 0.18	77.27 ± 0.06	90.64 ± 1.77
5 %	76.62 ± 0.65	92.09 ± 0.58	69.00 ± 0.65	77.30 ± 0.47	91.38 ± 1.18
10 %	76.84 ± 0.50	92.53 ± 0.41	68.40 ± 0.63	76.18 ± 0.69	90.51 ± 0.34
PS					
1 %	80.39 ± 0.80	91.71 ± 0.85		77.61 ± 0.35	86.32 ± 3.83
2 %	80.31 ± 0.64	91.22 ± 1.28		77.18 ± 0.44	85.30 ± 0.03
5 %	78.08 ± 0.57	90.14 ± 2.50		78.01 ± 0.44	
10 %	76.27 ± 0.34	86.60 ± 0.78		75.64 ± 0.35	
* Skin donor: 45-year-old woman, abdomen; T2: 71.43 ± 0.72 °C, T3: 86.87 ± 1.10 °C (untreated SC)					
Δ Skin donor: 44-year-old woman, abdomen; T2: 70.96 ± 0.37 °C, T3: 83.51 ± 0.43 °C (untreated SC)					

In order to evaluate the interaction of SC with the surfactants alone, SC was also incubated with surfactant solutions (1-10 %). Solutions of SL and SLS caused an exothermic event of SC at about 90 °C, but no endothermic events at lower temperatures. PS in concentrations of 1 % up to 10 % offered an additional endothermic event of about 38.5 up to 46.8 °C. Independent of the SLS concentration, T3 transition of SC shifted to lowest temperatures by -11 K compared with PS and SL, which in contrast revealed slight concentration dependence (see Table 1). The SL solution caused a further endothermic shift of the T2 transition of SC, which did not show clear concentration dependence with regard to a minor shift of about -2 to -3 K. Furthermore another endothermic event at about 90 °C was observed after incubating both, nanosuspensions and surfactant solutions, with SC.

This may be attributed to a shift of T4.

In conclusion, the surfactant is likely to cause the major interaction with the stratum corneum lipid structure, both from the continuous phase of the nanosuspension and from the surfactant solution itself.

[1] Dahl K, Müller-Goymann CC. Characterizing Beeswax-Jojoba Oil Nanosuspensions with TiO2 as Inorganic Sunscreen
with Emphasis on Different Emulsifiers, Poster, 16. GD-Jahrestagung 2012, Berlin.
[2] Van Duzee BF. Thermal analysis of human stratum corneum. J. Invest. Dermatol. 1975; 65:404-408.
[3] Bouwstra JA et al. Effect of N-alkyl-azocycloheptane-2-ones including azone on the thermal behaviour of human stratum
corneum. Int. J. Pharm. 1989; 52:47-54.
[4] Barry BW. Mode of action of penetration enhancers in human skin. J Control Release. 1987; 6:85–97.
[5] Leopold CS, Lippold BC. An attempt to clarify the mechanism of the penetration effects of lipophilic vehicles with differential scanning calorimetry (DSC). J Pharm Pharmacol. 1995; 47:276–281.

Wissenschaftliche Posterausstellung: Poster 4

Monitoring the distribution of anionic surfactants in the stratum corneum by combined ATR-FTIR and tape stripping experiments

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Introduction

Anionic surfactants are recognized as skin irritants. Especially sodium lauryl sulphate (SLS) is known to diminish the barrier properties of the stratum corneum. Therefore, SLS is mostly replaced by its ethoxylated and milder analogon sodium laureth sulfate (SLES) [1]. Nevertheless, SLS is still widely used in personal care products.

Combined with a tape stripping procedure, the ATR-FTIR technique can be employed to detect exogenous substance in different layers of the stratum corneum [2]. However, this approach is rarely used since characteristic bands of exogenous substances appear in the less attention paid fingerprint region and mostly overlap with typical skin bands.

In this study, with the help of spectral subtraction of untreated from treated skin, it was possible to monitor the distribution of anionic surfactants in the stratum corneum without the need of deuterated compounds.

The suitability of this method of analysis was tested with solutions of either SLS or SLES in water. In addition, two commercially available hair shampoos were used in a consumer orientated washing procedure on pig ear skin, in order to investigate the effects of a brief exposure on the uptake of SLS and SLES into the stratum corneum.

Experimental methods

Formulations

15% aqueous solutions of either SLS or SLES were used for incubation of the skin samples. Water served as control.

In case of the washing procedure, two commercially available shampoos containing SLS or

SLES were tested.

Washing procedure
Appropriate cut porcine ear skin samples were rubbed with shampoo in circular motion for one minute. After careful cleansing under running water, the skin sample was blotted dry and tape stripped once. ATR-FTIR spectra were recorded and analyzed as described.

Combined ATR-FTIR and tape stripping experiments
Full-thickness porcine ear skin was incubated with the respective surfactant solution for one hour at 32°C. Spectra were recorded on a Tensor 27 (Bio-ATR I tool, Bruker Optics, Germany) and analyzed with the software OPUS 5.5. The uppermost layers of the skin were removed with 20 consecutive adhesive films (Tesa film crystal clear sticky tapes, Tesa AG, Germany). The pseudoabsorption of the pooled corneocytes fixed to the individual tapes was determined with the SquameScan®850A (Heiland electronic GmbH, Wetzlar, Germany). Due to dependency of the ATR-spectra intensity on the degree of contact between the crystal and the sample, the absorbance of interest was normalized against the amid II absorbance. A skin sample incubated with water was tape stripped in the same manner and served as control.

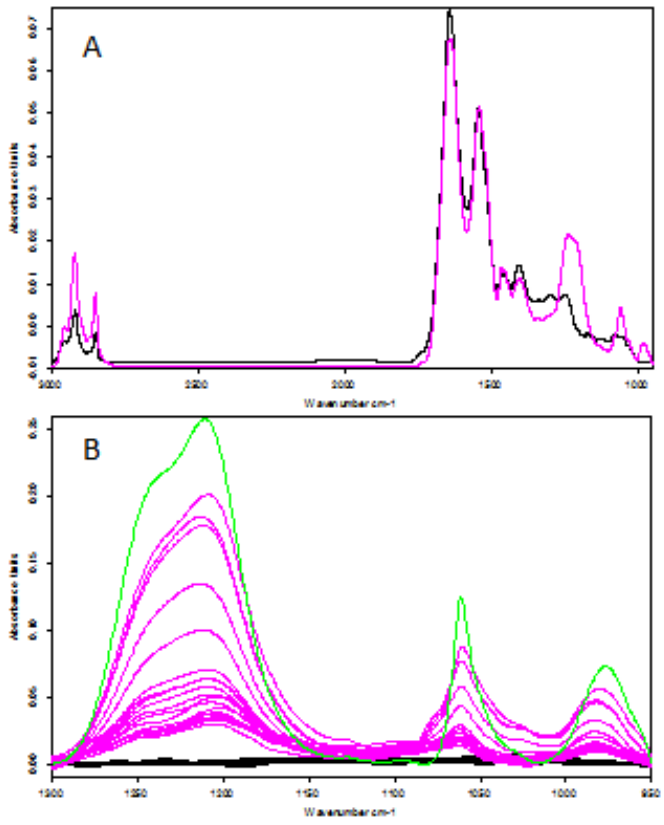

Figure 1:
(A) ATR-FTIR spectra of untreated porcine ear skin (black) and porcine ear skin incubated with SLS (pink); (B) Green: ATR-FTIR spectra of 15% SLS in water; Pink: ATR-FTIR spectra of SLS treated skin at different stratum corneum depth after subtraction of untreated skin Black: ATR-FTIR spectra after subtracting two different untreated skin samples from each other at different stratum corneum depth (control)

In order to monitor and compare the stratum corneum penetration depth of both surfactants, spectra of the control sample were subtracted from the treated sample. Due to different absorption coefficients of the evaluated absorption bands of SLS and SLES, the measured absorbances were additionally related to the corresponding absorbances of 15% aqueous solutions of SLS and SLES.

Results

Similar results were obtained after incubation with either SLS or SLES. As seen in Figure 1A for SLS, characteristic bands, like the alkyl sulfonate stretching bands at ~ 1210 cm-1, were an indicator of SLS incorporation into the stratum corneum. Subtraction of untreated skin spectra from SLS treated skin at equivalent depths resulted in spectra similar to those of the respective surfactant in water (Figure 1B). Both SLS and SLES were still detectable after removal of 20 tape strips, which corresponds to a stratum corneum thickness of about 50%. To prove the suitability of this method, we made a correlation of the deep-depended absorbances of two different absorption bands for both surfactants resulting in excellent linear correlations with coefficients of determination of 0.9956 for SLS and of 0.9863 for SLES, respectively.

With the help of this method, an uptake of SLS and SLES into the stratum corneum even after a short washing procedure with commercially available shampoos was observed.

References

[1] Charbonnier V, Morrison BM, Paye M, Maibach HI. Food Chem Toxicol 2001; 39: 279-286

[2] Schwarz JC, Pagitsch E, Valenta C. Eur J Pharm Sci 2013; 50 (5): 595-600

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Wissenschaftliche Posterausstellung: Poster 5

CMS nanotransporters for topical delivery of dexamethasone

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In order to overcome the main skin barrier, the stratum corneum, and to increase the dermal penetration of drugs, various nanoparticulate carrier systems were developed during the last decades. Of particular interest are hyperbranched polyglycerol-based nanoparticles such as dendritic core-multishell (CMS) nanotransporters. CMS nanotransporters consist of a polyglycerol core which is surrounded by a lipophilic inner shell and a hydrophilic outer shell. The special architecture enables the encapsulation of a wide variety of guest molecules [1] and can transport them to polar and nonpolar environments. Their efficacy in terms of topical drug delivery of hydrophilic [2] and lipophilic [3] guest molecules was demonstrated previously.

The glucocorticoid dexamethasone (DXM) is the gold standard for the topical therapy of inflammatory skin diseases. However, long term use is often accompanied by severe side effects such as skin atrophy. Loading of DXM onto CMS nanotransporter may reduce side effects as shown with liposomes [4].

Hence, in this study we established a method to determine the loading properties and loading capacity of the CMS nanotransporter for DXM using high performance liquid chromatography (HPLC). A RP-18 column, the eluent acetonitrile/water (40:60) with a 0.5 ml/min flow rate, an external standard and a detection wave length of 254 nm was chosen. Based on x-ray microscopy measurements, a DXM concentration of 5 % loaded onto CMS nanotransporters was calculated. Analyzing the HPLC data, we recovered about 3 % DXM. However, we only detect unloaded DXM. Considering the maximal solubility of DXM in water (80-100 µg/ml), we detected about 2-fold higher DXM concentration in the presence of CMS nanotransporters. This solubilization effect can be explained by the ability of the CMS to form spontaneous aggregates simultaneously encapsulating DXM [1]. However, these aggregates are not stable under strong shear stress as occurring in an HPLC (40 bar). Hence, the CMS nanotransporters disaggregate and release the DXM. Nevertheless, investigations using fluorescent life time imaging microscopy (FLIM) showed an encapsulation of hydrophobic cargos also in the unimolecular state clearly indicating that the loading properties for the CMS nanotransporters are highly drug dependent [5].

By combining different analytical methods such as FLIM and HPLC the loading properties of CMS nanotransporters can be unraveled most effectively demonstrating the diversity of the

CMS nanotransporters. More detailed studies on drug release and the dermal delivery capacity of DXM loaded CMS nanotransporters are currently under investigation.

Acknowledgement
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References

1.Quadir, M.A. and R. Haag, Biofunctional nanosystems based on dendritic polymers. J Control Release, 2012. 161(2): p. 484-95.
2.Küchler, S., et al., Influence of nanocarrier type and size on skin delivery of hydrophilic agents. Int J Pharm, 2009. 377(1-2): p. 169-72.
3.Küchler, S., et al., Nanoparticles for skin penetration enhancement--a comparison of a dendritic core-multishell nanotransporter and solid lipid nanoparticles. Eur J Pharm Biopharm, 2009. 71(2): p. 243-50.
4.Fesq, H., et al., Improved risk-benefit ratio for topical triamcinolone acetonide in Transfersome in comparison with equipotent cream and ointment: a randomized controlled trial. Br J Dermatol, 2003. 149(3): p. 611-9

Wissenschaftliche Posterausstellung: Poster 6

Laser diffractometry size analysis of nanoparticles: the ignored pitfall of refractive indices!

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Laser diffraction (LD) is one of the most frequently used methods for particle size analysis of nanomaterials. The measuring range is 20 nm-2000 µm, i.e. it allows also detection of aggregates in the µm size range besides the nano bulk population. In the range 20 nm to about 4 µm the size needs to be calculated using the Mie theory which requires input of the real refractive index (RI) and imaginary refractive index (IRI). Both indices are material specific. Often the indices are not known, especially the RI, and values were estimated or the set value in the program was used. This leads to an article stating that 90% of the published measurements are false [1].

In this study, lipid nanoparticles (SLN, NLC) were used to assess the extent of error which can occur. They are ideal for such a study because of ease of exchange of core material, stabilizer layer and modification of size. For the last 10 years, the published nanoemulsions RI value of 1.456 combined with IRI of 0.01 were used for LD size calculation of many differently composed solid lipid nanoparticles. This can potentially lead to wrong calculation of size results. In this study it was systematically analyzed to which extent the chemical nature of the nanoparticle core material and the stabilizer layer, but also the particle size affect the RI, and consequently the calculated size in LD analysis.

Lipid nanoparticles were produced using three different core lipids (Cutina CP, Compritol 888 ATO and Dynasan 116), stabilized by three surfactants (Tween 80, TegoCare 450 and SDS), yielding a total of 9 different formulations (= solid lipid nanoparticles = SLN). Another 9 formulations were produced by loading these lipid particles with argan oil as cosmetic active (= nanostructured lipid carriers = NLC). The RI was measured by analysis of variously diluted lipid particle suspensions (e.g. 1-10%) using an Abbé refractometer at 200C [2], and extrapolating the RI to a solid content of 100%. The IRI was determined by fitting the originally detected scattered light data with the scattering curve analyzed by the software of the instrument (i.e. equal values at channel 100). All LD measurements were performed by a Mastersizer 2000 (Malvern Instruments Ltd., UK).

Both the core lipids and the stabilizers in the formulations were found to distinctly affect the RI. For example: RI values of 1.493, 1.514 and 1.488 were obtained, when using aragan-oil loaded Cutina CP NLC stabilized with 3 different surfactants (Tween 80, TegoCare 450 and SDS). Similar effects were observed for the other two lipids, confirming the strong influence of the stabilizer layer. Changing the lipid core composition to SLN (no argan oil) lead to a

decrease in the RI values for all nanoparticles. For example, for the Cutina CP-TegoCare 450 formulation the RI decreased from 1.514 to 1.464. Totally different particle diameters and size distributions are calculated with the wrong RI, e.g. the wrong RI of 1.456 yielded a diameter D99% of 0.892 µm and a monomodal size distribution, versus with the correct RI a diameter D99% of 3.106 µm and a bimodal size distribution were obtained. The large peak representing the relatively large particles (aggregates) in the sample. The RI is mainly affected by the chemical nature of the components. In contrast, the IRI showed no or little dependence on component but on particle size. For nanoparticle samples studied, IRI in the range 0-0.01 yielded no big differences in calculated size and size distributions.

For all nanomaterials it can be concluded, that chemical composition of core material but also composition of the stabilizer layers affects strongly the RI. Measurement of the RI is therefore an essential prerequisite for correct size analysis. Differences in RI of about 0.01 and larger can have a significant effect on the calculated size (rule of thumb). The diameters 90%-99% are much more affected than the diameters 50% in case a wrong RI is used. For the determination of IRI, the instrument software appears suitable.

References:

[1] Keck, C. M., Müller, R. H., Size analysis of submicron particles by laser diffractometry – 90 % of the published measurements are false, Int. J. Pharm. 355, 150-163, 2008
[2] Cornelia M. Keck, Cyclosporine Nanosuspensions: Optimized Size Characterization & Oral Formulations, Berlin, Free University of Berlin, 2006
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Wissenschaftliche Posterausstellung: Poster 7

Improved solubility properties oftopical azithromycin nanocrystalsfor prophylaxis of borreliosisinfection

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The worldwide rate of Lyme disease infection has been increasing in recent years [1]. Among several defenses against it, using antibiotics is the most effective and economical approach [2]. Usually oral antibiotics are administered to patients who have been bitten by ticks. However the systematical administration of antibiotics could lead to some side effects such as bacterial resistance. Thus topical formulation for prophylaxis of Lyme disease infection is in demand.

Dermal azithromycin formulations have already been demonstrated efficacy and safety in clinical phase 1 and 2 studies [1-3]. Drug concentrations required being up to 10% [3]. However, the application of raw drug powder [2] limits bioavailability in the skin due to slow dissolution velocity of µm-sized crystals. Therefore in this study, the solubility properties of azithromycin were investigated when formulated as nanocrystal suspension (nanosuspension). Nanocrystals possess not only a higher dissolution velocity than large crystals, but also a higher saturation solubility, thus creating a higher concentration gradient and increasing skin penetration.

Azithromycin nanocrystals with a size of 189 nm were produced by bead milling, stabilized in suspension with tocopheryl polyethylene glycol succinate (TPGS). The saturation solubility of the nanosuspension compared to raw powder was determined in water by shaking for 8 hours in vials; dissolution velocity was determined by measuring dissolved drug concentrations as a function of time. The drug concentrations were analyzed by HPLC. The nanosuspension had an about 2 times higher saturation solubility in water (227 µg/ml) compared to the raw drug powder.

To create an even higher concentration gradient with the nanocrystal formulation, nanocrystals were dispersed in a water-propylene glycol mixture (80:20, w/w). The saturation solubility of the nanocrystals increased to 2828 µg/ml. In addition, the dissolution velocity was much higher for the nanocrystals than for the raw drug powder. Compared to their respective saturation solubility, the nanocrystals dissolved to 99% within 20 minutes and the raw powder showed only 68% dissolution even after 2 hours.

In summary, fast dissolution and increased saturation solubility could be shown for the azithromycin nanosuspension. Based on nanocrystal theory - it is expected that the drug concentration of 10% [3] in the dermal formulation can be distinctly reduced due to the higher thermodynamic activity of the nanocrystals, at simultaneously higher drug concentration in the skin.

References:

[1] Huber, G., Hüttenes, U., Bidder, R. C., USP 0130437, 2010
[2] Piesman, J., Hojgaard, A., Ullmann, A. J., Dolan, M.C., Antimicrob. Agents Chemother. 58 (1), 348-351, 2014
[3] Knauer, J. et al., J. Antimicrob. Chemother. 66 (12), 2814-2822, 2011

Wissenschaftliche Posterausstellung: Poster 8

The antioxidant status of the human skin: A comparison between South Korea and Germany

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Background: Carotenoids can serve as marker substances for the antioxidant status of the skin providing protection against the destructive action of reactive oxygen species. Since most antioxidants cannot be produced by the human organism, they have to be ingested with a nutrition rich in fruit and vegetables. The traditional Korean cuisine is largely based on uncooked vegetables which can provide an increase in antioxidants, especially carotenoids, but little is known about differences in the antioxidant status of Western and Asian populations.

Objective: In this German-Korean study, we investigated whether dietary differences between German subjects, South Korean subjects and Korean immigrants to Germany are reflected in the cutaneous antioxidant status considering the different dietary and socio-cultural factors in South Korea and Germany.

Methods: The carotenoid concentrations of 279 Korean volunteers resident in South Korea, 332 German volunteers resident in Germany and 103 Korean-German immigrant volunteers resident in Germany were measured and individual data regarding lifestyle and dietary habits were analyzed. Measurements were performed non-invasively on the skin of the hand palm using a mobile measuring system, based on reflectance spectroscopy.

Results: The mean carotenoid concentration of the Korean subjects in South Korea was shown to be significantly higher (5.81±0.11; $p<0.001$) than the mean concentration of both the German subjects (4.62±0.10) and of the immigrant Korean subjects in Germany (4.77±0.18). Furthermore, the Korean-born first generation of immigrants had come during the 1960's and 1970's to Germany and mostly preserved Korean dietary habits, showing significantly higher

mean concentrations ($p<0.001$) than the German born second and third Korean generations in Germany.

Conclusion: The results of the study demonstrate that the higher uptake of antioxidants in Korean subjects is reflected in a higher antioxidant status. It also indicates that not only a healthy nutrition but also a simultaneously low stress exposure are essential to obtain a high antioxidant status.

Wissenschaftliche Posterausstellung: Poster 9

Treatment of cutaneous T-cell lymphoma with alitretinoin

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Background
Cutaneous T-cell lymphoma (CTCL) is a potentially life-limiting malignant disease. Treatment strategies in CTCL aim at disease control and remission with the lowest possible side effects. Recent reports suggest that the new vitamin A derivative alitretinoin might be a well-tolerated treatment option.

Patients and Methods
We analysed the files of 13 CTCL patients with mycosis fungoides (n=11) or Sézary syndrome (n=2) who were treated with oral alitretinoin alone or in combination with standard treatment based on individual off-label treatment decisions. Patients had been monitored every 4-8 weeks with skin examination and laboratory analyses.

Results
The largest proportion (84.6%) of patients was classified as early stage disease (IA: n=6, IB: n=4, IIB: n=1). 15.4% had already progressed to advanced disease stages (IIIA: n=2). 12 of 13 patients (92.3%) showed a marked improvement of their CTCL skin lesions and no progress of the disease, only 1 patient showed no response to the treatment (7.7%). 5 of the responding patients (41.7%) achieved a complete response and 7 (58.3%) experienced a partial response. Average time to response was 2.6 months. Duration of treatment varied depending on whether patients had reached complete or partial remission. In general, alitretinoin was well tolerated. One patient developed high non-fasting average serum cholesterol (> 300 mg/dl) and a mean non-fasting triglyceride value > 500mg/dl. In 4/13 patients, thyroid-stimulating hormone declined without clinical symptoms during treatment, with 1 of the patients also showing a decreased thyroxin level.

Conclusions
In our cohort of CTCL patients we noticed a low rate of side effects and an overall good clinical response to treatment with alitretinoin, making this novel retinoid a promising alternative to established therapies for CTCL. Further studies are required to substantiate this early clinical observation.

Wissenschaftliche Posterausstellung: Poster 10

Effects of INLB321-CD on VEGFA gene up-regulation in immortalized human keratinocytes and on a model of wounded skin

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Internalin B (InlB) is an invasion protein of Listeria which facilitates its uptake into host cells by activating the receptor tyrosine kinase MET. It was proposed that activation via receptor dimerization is mediated by an InlB dimer. The dimerized fragment of Internalin B, InlB321-CD[1] (crystal dimer), was designed to stabilize the InlB dimer in solution. In binding studies and in in vitro scatter assays[1], InlB321-CD revealed to be a stronger agonist than monomeric InlB321 and Internalin B.

In human skin, mainly epithelial cells express the MET receptor whose activation leads to proliferation, migration and vascularization. Its endogenous agonist hepatocyte growth factor (HGF/SF), which is secreted by e.g. dermal fibroblasts, plays an important role in the regeneration of the epidermis of the skin. That is why in preliminary studies, InlB321-CD was investigated with focus on its mitogenic and motogenic properties2,3 on human epidermal immortalized cell line (HaCaT).

The present study aims at InlB321-CD's influence on the up-regulation of the vascular endothelial growth factor (VEGFa) at RNA level, because HGF/SF stimulates vascularization via secretion of VEGF4. In vivo, particularly in chronic wounds, HGF/SF is degraded by proteases causing retarded vascularization.

Furthermore, InlB321-CD's effect on a mechanically wounded and differentiated epidermis model was analyzed in terms of its wound healing properties.

Methods:
A confluent HaCaT monolayer was serum-starved (24 h), then incubation with serum-free medium, 0.5 nM HGF, 0.5 nM InlB321-CD and 1 nM InlB321 took place for 6 h. Total RNA was extracted with Trizol© according to the manufacturer's guidelines. RNA concentration was quantified with an UV spectrometer. Prior to performing PCR with a pair of gene specific primers for VEGFa, first strand DNA synthesis was carried out. The PCR products were separated with an agarose gel electrophoresis stained with ethidium bromide and detected under UV light (260 nm).

HaCaT cells were cultivated on a polycarbonate membrane (3µm pore size) which was set on a dermis consisting of living fibroblasts incorporated in a collagen matrix5. After 3 weeks of co-culture, 3 days of serum-starvation was conducted. Then the membrane with the differentiated epidermis was removed. Subsequent to perforating the epidermis with a punch, cultivation on a dermis with either dead or living fibroblasts was conducted for further 5 days in serum-free medium with or without 0.5 nM InlB321-CD supplementation. An MTT assay was used to test for viability of the epidermis.

Results:
The VEGFa gene expression at RNA level after incubation with 0.5 nM InlB321-CD was slightly higher compared with medium control, and comparable to 0.5 nM HGF/SF, which served as positive control. However, the equimolar dose of monomeric InlB321 did not increase VEGFa gene on mRNA level.

Subsequent to incubation with 0.5 nM InlB321-CD, the epithelial model of wounded skin co-cultured on a dead dermis showed a higher relative cell viability compared to that treated with plain medium. In contrast to that, the differentiated keratinocytes co-cultured on a living dermis did not benefit the same way from 0.5 nM InlB321-CD supplementation versus medium. This might be due to fibroblasts' secretion of growth factors, i.e. HGF/SF that endogenously stimulates the mitogenic process in keratinocytes.

In conclusion, for proof of wound healing potential of InlB321-CD treatment of a wounded 3D skin model, the endogenous HGF/SF secretion of fibroblasts from the dermal layer underneath the epidermal layer has to be suppressed.

References:
[1] Ferraris, D.M. et al., Ligand-Mediated Dimerization of the Met Receptor Tyrosine Kinase by the Bacterial Invasion Protein InlB, J. Mol. Biol. (2010), 395, 522-532
[2] Kolditz, F. et al., Poster presented at DPhG-Jahrestagung, Braunschweig (2010), http://www.digibib.tu-bs.de/?docid=00038117
[3] Kolditz, F. et al., Poster presented at APV meeting, Istanbul (2012)
[4]Gille, J. et al., Hepatocyte Growth Factor/Scatter Factor (HGF/SF) Induces Vascular Permeability Factor (VPF/VEGF) Expression by Cultured Keratinocytes, J Invest Dermatol. (1998), 111, 1160-1165
[5] Hoffmann, C. et al., Use of artificial skin constructs in permeation studies of clindamycin phosphate. Pharmazie (2005), 60, 350–353

Wissenschaftliche Posterausstellung: Poster 11

Follicular penetration of a novel template of solid lipid microparticle dispersion for retinoids

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Introduction: Retinoids are highly teratogenic active pharmaceutical ingredients and cause severe congenital defects to the unborn child or spontaneous abortion. Thus, a novel formulation promoting the penetration of retinoids such as adapalene into the orifices of hair follicles as the drug target and enabling the circumvention of any systemic adverse effects meets an unmet medical need. Galenical target parameters are a mean particle size (MPS) of approximately 5 µm, a narrow particle size distribution, lipophilic material, and solidness of the particulate carrier system [1, 2].

The innovative pharmaceutical solid lipid microparticle dispersion (SLM) consisting of hydrogenated palm oil, purified phosphatidylcholine, poloxamer 407 (P407), polyethylene glycol 12000, potassium sorbate, anhydrous citric acid, and water in double distilled quality was optimized with a Box Behnken design for MPS and span in dependence on introduced lipid matrix (LM) content, P407 content, applied dispersion rate, and dispersion time. Potential for follicular penetration was evaluated via differential tape stripping on porcine ear skin compared to the commercial product Differin® cream [3].

Methods: LM and P407 phases were dispersed with different dispersion rates for varying dispersion times set within the design of experiments. MPS and span were determined with a laser diffractometer including polarization intensity differential scattering (Beckman Coulter, D-Krefeld). Approximately 30 mg SLM comprising 0.1%(w/w) adapalene and Differin® cream were spread on 6 cm^2 of the marked areas of the dorsal side of porcine ears for 3 minutes. 10 tapes (Beiersdorf, D-Hamburg) for tape stripping and 2 subsequent tapes for differential tape stripping with cyanoacrylate glue (Uhu, D-Bühl) were removed and extracted in the mobile phase of 45%(v/v) acetonitrile, 35%(v/v) tetrahydrofuran, and 20%(v/v) purified water for drug quantification via high performance liquid chromatography with a LiChroCART® 250-4 Purospher® STAR RP-18 endcapped (5 µm) column (Merck, D-Darmstadt), the mobile phase additionally containing 0.1%(v/v) acetic acid, a flow rate of 1.2 ml/min, and at a detection wavelength of 270 nm. Tapes were also analyzed via fluorescence microscopy (Olympus, D-Hamburg).

Results: The response surface designs of the constructed Box Behnken model reveal that the MPS decreased from about 4.6 to 3.5 µm with a lower content of LM,

a higher amount of P407, and a high dispersion rate no matter what dispersion time is applied. The narrowest span below 1.4 or 1.2 µm, respectively, was obtained with a LM content up to 20%(w/w) with 12%(w/w) P407 as the phase parameters while using a dispersion rate of 16000 rpm and dispersion time of 3 minutes as the centrally set process parameters.

Both the optimized SLM loaded with 0.1%(w/w) adapalene and the Differin® cream exhibited a high density of single fluorescent signals on the first 2 tapes being indicative of the presence of lipid particles containing adapalene or adapalene crystals, respectively, on the surface. Isolated hair follicles on the 2 cyanoacrylate tapes featured the particular signals as well, demonstrating a penetration into the orifices of hair follicles of both formulations.

2.031 µg adapalene/cm² from the first 10 tapes for the applied SLM and 1.509 µg adapalene/cm² from the tapes for the Differin® cream were detected which may be assigned to the stratum corneum [4]. Regarding the follicular content, 0.257 µg adapalene/cm² from the SLM and 0.117 µg adapalene/cm² from Differin® were recovered from the cyanoacrylate tapes. No statistically significant difference between both contents was determined. However, the absolute amount of penetrated adapalene was higher for the SLM.

In conclusion, the novel optimized pharmaceutical formulation provides a similar penetration behaviour like a commercial dermal product and does actually show a follicular penetration.

Disclosure: The authors filed a patent application for the novel pharmaceutical formulation claiming a broad range of the composition.

References:

[1] Schaefer, H., Skin Pharmacol. (1993), 6(1), 17-23.
[2] Toll, R., et al., J. Invest. Dermatol. (2004), 123(1), 168-176.
[3] Teichmann, A., et al., J. Invest. Dermatol. (2005), 125(2), 264-269.
[4] Lademann, J., et al., Eur. J. Pharm. Biopharm. (2012), 80(3), 615-620.

Wissenschaftliche Posterausstellung: Poster 12

Ultra-small nanoparticles promote thepenetrationof CoQ10inthe skin – a counteract against oxidative stress

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The exterior layer of the skin protects against negative environmental influences and (UV-) radiation. UV radiation leads to the formation of free radicals, in particular the formation of reactive oxygen species (ROS). ROS are produced in the whole organism and are thus in equilibrium with the antioxidant systems of the body which consists of enzymatic and non-enzymatic antioxidants. If the antioxidant system is disturbed, cell damage, premature skin aging and the development of skin cancer can occur. To counteract these processes antioxidants are contained in many cosmetic products and sunscreens, such as the coenzyme Q10 (CoQ10, alternative name: Ubiquinon). CoQ10 is a lipophilic non-enzymatic antioxidant, produced by the body itself and is localized in the mitochondrial membrane. In its reduced form (ubiquinol) CoQ10 has a high antioxidant potential, scavenges free radicals and thus protects against oxidative stress and leads to minimization of cell damage. Free CoQ10 is sparingly soluble in water and cannot penetrate into cells. In recent years, lipid nanoparticles have been developed to facilitate the penetration of lipophilic molecules into the skin. NLC (nano structured lipid carriers) represents a good basis for transporting molecules into the cell. In order to further improve and optimize the penetration efficiency, ultra-small nanoparticles (usNLC) of < 100nm in size were developed. They consist of a solid lipid core and a sheath of liquid lipid in which the active ingredient (CoQ10) is mainly localized. Their small size (85nm) should facilitate the uptake of agents into the cell, thereby increasing the bioavailability of the drug. This was investigated using fluorescence microscopy on nile red loaded usNLC. To test the antioxidant effect, CoQ10-loaded usNLC were analyzed via electron paramagnetic resonance spectroscopy (EPR) in HaCaT keratinocyte cells which were exposed to UVA/B radiation (1J/cm^2/ 18mJ/cm^2), triggering the formation of free radicals. The non-toxic CoQ10 concentration was evaluated via a cell viability test (XTT cell proliferation assay), determining the viability of the cells which is proportional to the number of living cells.

The XTT assays revealed that CoQ10 concentrations of 10, 25 and 50 µg/ml show no significant effect on the cell viability; in contrast to 100 µg/ml which results in a strong limitation of the cell viability. Therefore, the antioxidative effect of usNLC was examined on HaCaT cells using the concentrations 10 to 50µg/ml of CoQ10. For UVA a higher radical formation could be detected, UVB radiation is indeed more energetic but will damage more cell

compartments and structures. The EPRinvestigations with usNLC-CoQ10 demonstrated a clear reduction of the radical formation of up to 9% in UVA irradiated cells compared to control but no dependence on usNLC-CoQ10 concentration. Using fluorescence microscopy, the penetration of the loaded usNLCs into the cells could be shown.

In this study, it could be demonstrated that CoQ10 loaded usNLC penetrated into HaCaT cells and showed an antioxidant potential. These particles are a further development of the previously analyzed nanoparticle systems and represent a new era of nanocarrier systems.

Wissenschaftliche Posterausstellung: Poster 13

Investigation of the penetration of vehicle components and active drug from fluorosurfactant-based microemulsions

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Introduction

Microemulsions are established formulations for transdermal drug delivery, but the mechanisms and factors controlling their transdermal drug penetration enhancement are still not clear. Since the components of a microemulsion are known to facilitate drug transport across the barrier it was the objective of the present study to monitor the penetration route of the incorporated drug and the fluorosurfactant as specific vehicle component and to examine whether synergies arise regarding their stratum corneum uptake. To this end, the penetration depth of each compound was elucidated through tape stripping studies via simultaneous quantification of diclofenac-sodium (DS) and the fluorosurfactant. The active component (DS) as well as the fluorosurfactants were directly quantified from the same strip by HPLC and 19F NMR, respectively. Moreover, ATR-FTIR experiments with the formulations and pure fluorosurfactants were performed to elucidate their effects on skin integrity.

Experimental methods

Skin penetration experiments

Tape stripping studies were carried out on porcine ear skin with an oleic acid solution incorporated with 0.7% (w/w) diclofenac-sodium as control and with two selected microemulsions containing the fluorosurfactants Hexafor 670 (Hex) or Chemguard S-550-100 (Sin), isopropyl alcohol as co-surfactant in the relation 1:1 (w/w), oleic acid as oily component, distilled water and 0.7% (w/w) diclofenac-sodium. 5 mg/cm^2 of formulation were applied and after 1 h exposure the stratum corneum layers of the porcine ear skin were removed by Corneofix® tapes. This procedure was repeated for each individual tape stripping experiment until the entire stratum corneum was stripped off. The tape strips were then immersed in 2 mL CH3OD. Each tape strip was quantified by HPLC and 19F NMR. The quantified cumulative amounts of both components were further used for calculation of their relative slopes in order to examine whether any relation exists between their penetration behaviours.

19F NMR

NMR spectra were recorded on a Bruker Avance III 600 NMR spectrometer (Bruker BioSpin GmbH, Germany) operating at 564.69 MHz for 19F. A 5-mm quadruple observe probe

equipped with z-axis gradient coil was used. All measurements were performed in deuterated methanol at a temperature of 298 K. Other typical acquisition parameters chosen were: 15 ppm spectral with, 32 000 data points, 90° excitation pulse, 2 s acquisition time and 1000 scans. As an external reference to calibrate the 19F, the chemical shift scale CCl3F was used. The processing and the analysis of the NMR spectra were performed within the Topspin Software (version 3.0; Bruker BioSpin GmbH).

ATR-FTIR studies

Infrared spectra of porcine ear skin samples were obtained using a Tensor 27 FTIR instrument (Bruker Optics, Germany) equipped with a Bio-ATR I tool at the skin surface temperature of 32°C. The skin samples were placed surface down on the ZnSe ATR crystal. ATR-FTIR spectra were recorded before and after impregnation with a formulation.

Results

The curve shapes of the calculated relative slopes of diclofenac-sodium and fluorosurfactants from the microemulsions proceeded almost equal, but completely different to the DS-curve from the oleic acid solution.

Both microemulsions as well as the oleic acid solution provoked significant changes in the absorbance spectra of the SC ($P < 0.05$). Interestingly, after the application of pure fluorosurfactants no shifts of the CH2 stretching bands could be detected.
On the one hand the FTIR-results indicated a penetration enhancement of diclofenac-sodium due to a conformational disorder of the SC lipids induced by oleic acid, but on the other hand the nearly identical slope curves of diclofenac-sodium and fluorosurfactant from the microemulsions also suggested an influence of the employed fluorosurfactants. However, the shift of the CH2 stretching absorbances only occurred when the fluorosurfactants were part of the microemulsion systems. Apparently, their combination and the arising microstructure of the prepared microemulsions exerted specific effects on skin integrity resulting in an enhanced diclofenac-sodium penetration.

Acknowledgements

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Wissenschaftliche Posterausstellung: Poster 14

Nanocrystals – a “needle free injection” of drug into the skin

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In 2007 the first cosmetic product containing nanocrystals was introduced by Juvena Switzerland with line JUVEDICAL and other products such as e.g. platinum rare from la prairie followed. Nanocrystals are particles made of pure cosmetic or pharmaceutical active, having a size in the nanodimension (few nm to < 1,000 nm). Dermal penetration enhancement takes place theoretically by 3 effects:

1. increased saturation solubility (thus increased concentration gradient) and
2. increased dissolution velocity, both compared to micrometer-sized powders
3. high adhesion to skin

The literature reports increase in penetration and distinct increase in bioactivity. For rutin nanocrystals an increase in the antioxidant activity in the skin was reported by a factor 1,000. The rutin nanocrystals were 2 x more effective in increasing the sun protection factor (SPF) in a human irradiation study at only 1/500 concentration of dissolved active [1].

Within the study a formulation against couperosis was developed containing rutin nanocrystals and nanostructured lipid carriers (NLC) loaded with vitamin K1 and vitamin A1. NLC are also described having a penetration enhancing effect by forming an occlusive film.

A direct comparison of rutin nanocrystals to rutin NLC could not be performed, due to the fact that rutin is poorly soluble in lipids. Only comparison of release of lipophilic actives in different carrier systems was possible. This should give evidence which carrier might be more efficient in dermal delivery. Therefore the penetration form rutin nanocrystals and vitamin A1 loaded NLC were compared by performing an ex vivo tape stripping test.

The results obtained were shown by penetration profiles (fig. 1 and 2) plotting the concentration in the strips (µg/ml) versus the relative stratum corneum thickness (%).

After 20 minutes, the rutin nanocrystals show a very pronounced penetration as indicated by the high concentrations visualized by horizontal bars, especially in the upper 60% of the stratum corneum. It clearly indicates superior penetration enhancing effects compared to NLC.

Based on short exposure time and high concentration observed, the nanocrystals resemble an injection or micro-needle like penetration behavior.

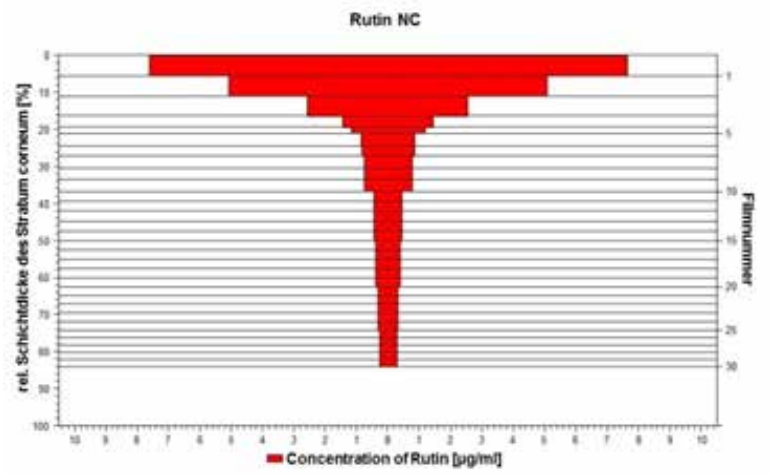

Fig. 1: Penetration profile of rutin nanocrystal suspension on pig ear skin after 20 minutes application time

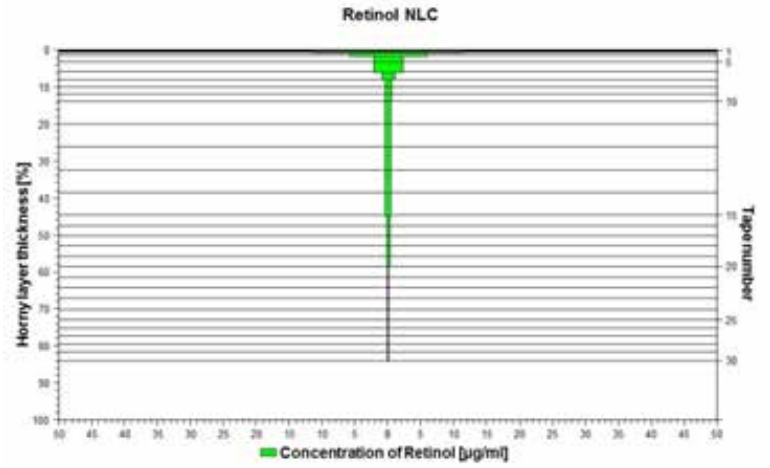

Fig. 2: Penetration profile of vitamin A1 NLC suspension on pig ear skin after 20 minutes application time

Very interesting was - against all expectations - that the combination of rutin nanocrytals and NLC did not further improve rutin penetration. On the contrary addition of NLC slightly reduced rutin penetration. This is an important finding for future selection of formulation compositions.

[1] Petersen, R.D., PCT/EP2007/009943, 2006, Abbott GmbH Germany

Wissenschaftliche Posterausstellung: Poster 15

Ultra fine cyclosporin A nanocrystals produced in a super small scale

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Cyclosporin A (CyA) is a potent imunosupressor used in case of organ transplants and also for dermal diseases such as psoriasis. Its administration via oral has many drawbacks, including toxicity, systemic effects and low bioavailability. In case of dermal diseases, the topic route would be recommended. However, cyclosporin A has very low skin penetration and consequently, low dermal bioavailability and bioactivity. One approach to overcome these problems is formulating it as nanocrystals [1]. Size below 100 nm is essential, because it has the highest increase in saturation solubility. This creates largest penetration-enhancing concentration gradient between dermal formulation and skin. Therefore, in order to increase the topical performance of this drug, ultra fine CyA nanocrystals in the lower nanorange (<100 nm) were produced in a super small scale (0.5 g batch).

The production principle was a top-down approach, in this case, wet bead milling in a super reduced scale. The milling chamber consisted of a 1 mL glass vial filled with 50% (v/v) grinding media and 50% (v/v) CyA coarse suspension. It was processed in a magnetic stirrer at 1,200 rpm and 5°C for 5 days. Particle size was assessed by photon correlation spectroscopy (PCS) and light microscopy. Samples were drawn after 1hour, 6 hours, 1 day, 2 days, 3 days and 5 days.

Up to 3 days milling, particle size gradually reduced as a function of time. The smallest PCS diameter was 93 nm and polydispersity index was 0.138. Such super small scale production is meaningful for the development of formulations of high costly drugs and new chemical entities which are not available at large amounts. For instance, the batch size of a traditionally used bead mill such as the PML-2 (Bühler AG, Switzerland) is normally around 150 g for the discontinuous mode. In the super small scale investigated in this study, the batch size is reduced by a factor of 300 fold.

CyA nanocrystals <100 nm for enhanced skin penetration were successfully produced and a super small scale approach for production of nanocrystals was established. This is meaningful for the R&D of formulations of new and/or high costly molecules e.g., radiolabeled drugs for pharmacokinetics studies.

[1] Shegokar, R., Müller, R. H., Int. J. Pharm. 399, 129-139, (2010)

Wissenschaftliche Posterausstellung: Poster 16

Soft matter characteristics of topical poloxamer 407-based formulations – the influence of temperature and ibuprofen content

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Dermal application of non steroidal anti inflammatory drugs (NSAID) is a popular and effective alternative in the short term treatment of pain and inflammation in the muscles and joints reducing gastro intestinal side effects which are a major problem with peroral administration [1]. Poloxamer 407-based formulations with the NSAID ibuprofen (IBU) have already shown high in vitro permeation rates across isolated human stratum corneum [2]. In addition to an adequate drug permeation across skin, rheological properties (e.g. consistency, yield stress) are of great importance to promote comfortable skin application. Therefore, a model formulation containing 24 % poloxamer 407 (POX), 6 % medium chain triglycerides (MCT), 10 % isopropanol (IPA), 20 % dimethyl isosorbide (DMIS), and 40 % water was analyzed with regard to the influence of the ibuprofen content (0, 5, 10, and 14 %) on the rheological characteristics.

Methods: Manufacture of the formulations was performed with a Cito Unguator® 2000 (Konietzko GmbH, D-Bamberg). Rheological studies were performed with a HAAKE Rheo Stress 6000 rheometer (Thermo Fisher Scientific, D-Karlsruhe) equipped with a cone/plate (1 °, 20 mm) shear apparatus. Rotation mode was used to determine flow curves and yield stresses. Since poloxamer-based gel formulations may show thermoreversible gelation [3], the measurements were performed at different temperatures including body and ambient temperature (37 °C and 20 °C, respectively). As pain reduction may be supported by a cooling effect of the vehicle, refrigerator temperature (5 °C) was tested as well. Oscillatory mode was used to investigate viscoelastic properties of the samples. Measurements were performed at 25 °C and 40 Pa (linear viscoelastic range for IBU-free formulation). Complex viscosity at a frequency of 0.5 Hz was determined to describe consistency of the systems. The microstructure was analyzed with a polarizing microscope (Leica DMLM, Leica Microsystem GmbH, D-Wetzlar) equipped with a lambda plate.

Results: The polarizing micrographs showed significant differences in the microscopical appearance of the formulations. Systems with 0 and 5 % IBU were isotropic, those with 10 and 14 % showed anisotropic textures (hexagonal and lamellar, respectively). The changes in microstructure corresponded with an alteration of the rheological properties. 5 % IBU increased the yield stress of the formulation along with its consistency, whereas a further increase in IBU content up to 10 % led to a softening. In contrast, 14 % IBU produced the highest value for complex viscosity despite the lowest yield stress. None of the systems showed thermoreversible

gelation within the temperature range studied. Yield stresses of IBU-loaded formulations decreased with increasing temperature.

Conclusion: IBU as an amphiphilic molecule interacted with the excipients so that a variation in IBU content led to significant changes in microstructure and rheological properties of the formulations. The results illustrate that rheometry is an appropriate method to detect structural changes in semisolid drug delivery systems.

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References:

1. Massey T, et al. Topical NSAIDs for acute pain in adults. Cochrane Database Syst Rev 2010; (6): CD007402.
2. Schmid S, Müller-Goymann CC. Topische Schmerztherapie - Auf die Galenik kommt es an! (Topical NSAIDs - On the importance of the vehicle). Aktuelle Derm 2012; 38(12): 499-504.
3. Schmolka IR. Artificial skin I. Preparation and properties of pluronic F-127 gels for treatment of burns. J Biomed Mater Res 1972; 6(6): 571-582

Wissenschaftliche Posterausstellung: Poster 17

Dermal formulations with rutin nanocrystals & peptide-loaded liposomes - mechanisms & in vivo performance

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Drugs soluble in water or lipophilic media can be incorporated into liposomes, the novel delivery system nanocrystals is suitable for formulating poorly soluble actives. From this a combination of both delivery systems is able to deliver each cosmetic or pharmaceutical active mixture to the skin – independent on its solubility. This combination was firstly realized on the market in cosmetic products (e.g. intense lifting eye serum, Dr. JK Cosmeceuticals).

Primary penetration enhancing physical mechanisms are occlusion for liposomes, penetration into the skin can only occur when using liposomes with special composition (transfersomes [1]). Nanocrystals are crystals in the nano dimension (typically 300-600 nm) which create an increase in saturation solubility for poorly soluble compounds. This leads to an increased concentration gradient between dermal formulation and skin, thus increasing passive diffusion. Active penetrated from the cream/gel into the skin is instantly replaced by new active dissolving from the nanocrystal depot, thus maintaining a constant high concentration gradient.

A formulation (intense lifting eye serum, Dr. JK Cosmeceuticals) was developed containing liposomes loaded with Argireline (acetyl hexapeptide-3) and Eyeseryl (acetyl tetrapeptide-5) and rutin nanocrystals. It was designed to improve the skin appearance and wrinkle profile around the eyes. The formulation was characterized in vitro regarding size characteristics (photon correlation spectroscopy, laser diffractometry), rheological behavior, and in vitro occlusion.

To assess in principle the in vivo skin effects in the eye region, this liposome-rutin combination [2] was investigated in a human study, male (8) and female (7) volunteers. The skin appearance was quantified using a VISIA Scan system (Canfield Imaging Systems, Fairfield, New Jersey), the skin profile (roughness) was quantified using the PRIMOS system, (GF Messtechnik GmbH, Teltow, Germany). Hyperpigmented area on the skin decreased significantly ($p<0.05$) in male and female groups after four weeks of treatment. Skin vascular structure was improved in the female groups ($p<0.05$). Thus the skin color was improved

because melanin and hemoglobin are the main skin colorants. Interestingly, skin roughness was more reduced in the female group after eight weeks of treatment. The roughness parameters decreased significantly ($p<0.05$), being most pronounced for the roughness parameter Rmax. There was a clear correlation between the decrease in roughness parameter Ra with the consumption of the product, highlighting the importance of application compliance to the skin effect.

References:
[1] Jain, S., et al., Drug Dev Ind Pharm, 2003. 29(9): p. 1013-26.
[2] Sinambela P., et al., Proceeding #900, Int. Symp. Control. Rel. Bioact. Mater. 40, Honolulu/Hawaii, 21-24 July 2013.

Wissenschaftliche Posterausstellung: Poster 18

Surfactant optimization for dermal azithromycin nanocrystals

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Azithromycin dermal formulations have been reported recently possessing better clinical efficacy than other topical antibiotics in the prevention of Lyme Borreliosis infection [1, 2]. The formulation in clinical testing is an ethanolic solution due to the poor water solubility of the drug. However, in general dermal suspension formulations are often superior to solution formulations, especially when nanosuspensions are used. Therefore an aqueous azithromycin nanocrystal formu¬lation was developed. In addition, ethanol evaporates after application, leading to the uncontrolled precipitation of drug as large particles. Smaller sized nanocrystals are also superior because of the smaller size and related larger surface area leading to a higher dissolution velocity in the moisture environment of the skin.

A two-step pearl milling method for the preparation of azithromycin nanosuspension was applied investigating six different surfactants, respectively: Poloxamer 188, Poloxamer 407, coco glucoside (Plantacare® 810 UP), decyl glucoside (Plantacare® 2000 UP), polyoxyethylene-20 sorbitan monooleate (Tween 80) and tocopheryl polyethylene glycol succinate (TPGS). 10% drug was dispersed in 1% surfactant solution by Ultra-Turrax for 1 minute at 8000 rpm. Then the resulting suspension was wet milled with yttrium-stabilized zirconia milling beads (size 0.1mm) until the particle size could not be reduced further. The process was performed at 5 °C.

For all formulations, independent on the surfactant, nanocrystals with diameters around 300 nm (z-ave) and a narrow size distribution (photon correlation spectroscopy polydispersity index around 0.2) could be obtained in just 10 minutes milling time. The nanosuspensions themselves were stable for 3 months. The optimized nanosuspension stabilized with TPGS with a size of 189 nm, PI 0.194, was incorporated into a 5% hydroxypropylcellulose (Klucel GF®) gel. The gel had the final concentration of 5% azithromycin described as being suitable for dermal Lyme Borreliosis treatment [2] and is by now physically stable for one month (no nanocrystal aggregation).

In summary, TPGS was found to be the most suitable surfactant for producing azithromycin physically stable nanosuspension. Nanocrystals below 200 nm were produced in a short production time. Production with a pearl mill can easily be scaled up, and the nanocrystals were also physically stable in the final gel formulation.

References:
[1] Piesman, J., Hojgaard, A., Ullmann, A. J., Dolan, M.C., Antimicrob. Agents Chemother. 58 (1), 348-351, 2014
[2] Knauer, J., Krupka, I., Fueldner, C., Lehmann, J., Straubinger, R. K., J. Antimicrob. Chemother. 66 (12), 2814-2822, 2011

Wissenschaftliche Posterausstellung: Poster 19

In vitro permeation behaviour and antimycotic efficacy of the antimycotic agent ciclopirox olamine incorporated into a variety of poloxamer 407-based formulations

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Introduction: Fungal nail infection (onychomycosis) is the most common nail disorder in adults and mostly induced by dermatophytes such as Trichophyton rubrum and Trichophyton mentagrophytes. Due to slow nail growth and depending on nail size and infected area, the treatment takes 3 – 9 months (1). To ensure a successful treatment, a highly effective active pharmaceutical ingredient (API) and a high penetration into and permeation through the nail is required in local therapy to maintain the drug concentration above the minimum inhibitory concentration (MIC).

In this study, the antimycotic agent ciclopirox olamine (CPX) was incorporated in concentrations up to 4 % (w/w) into poloxamer 407-based formulations and studied regarding its permeation behaviour through a keratin film as an artificial nail model. The poloxamer 407-based hydrogels consisted of poloxamer 407 (POX), double distilled water, propylene glycol (PG), isopropyl alcohol (IPA) and medium chain triglycerides (MKT). The marketed nail lacquer Ciclopoli®, containing 8 % ciclopirox as API, served as a reference.

Methods: The poloxamer 407-based formulations were weighed in an Unguator® jar and automatically stirred at 1440 rpm for 1.5 min with an Unguator® e/s GAKO Konietzko GmbH (Bamberg, Germany). To ensure sufficient equilibration, subsequent storage was done for 24 h at 20 °C. All the formulations were given codes reflecting their quantitative composition, e.g. 1P2525 represented a formulation loaded with 1 % CPX, while the vehicle itself contained 25 % POX/MKT (4:1), 25 % IPA/PG (1:1) and 50 % double distilled water (all w/w).

In vitro permeation studies (infinite dose technique) were carried out in modified Franz cells at 32 °C with 120 µm thick keratin films (KF) as permeation barrier. The receiver solution consisted of phosphate buffered saline (PBS) of pH 7.4. The quantification of the permeated drug amount was done with high performance liquid chromatography (HPLC) (Waters, Eschborn, Germany) by using a Grom-Sil 120 ODS 3-CP, 5 µm, 125 x 4 mm column (Grom, Herrenberg-Kayh, Germany) with acetonitrile/ethylenediamine tetraacetic acid disodium salt dihydrate solution (0.96 g/L)/acetic acid (600:400:0.1 (v/v/v)). The flow rate was set at 1.0 mL/

min; UV detection was done at 298 nm. A calibration curve ranging from 0.25 µg/mL to 200 µg/mL was recorded (r2 > 0.999) with a limit of detection (LOD) of 0.1255 µg/mL and a limit of quantification (LOQ) of 0.2510 µg/ml, respectively.

Infected nail plate studies analysing the antifungal activity were performed according to Lusiana et al (2).

Results: A variety of poloxamer 407-based formulations were analysed regarding the permeation behaviour as well as the antimycotic activity.

The vehicle P4030 showed a gel-like appearance with a ringing effect when the jar was knocked onto a hard surface. The saturation solubility was 5 % CPX. Saturation solubility was defined as the concentration with first appearance of API crystals under a polarising microscope Leica DM LM (Leica Microsystems GmbH, Wetzlar, Germany). With increasing drug concentrations, the poloxamer 407-based formulations became softer. Permeation studies through a keratin film as an artificial nail model showed that fluxes J (amount of permeated drug per area versus time) of the 2-4P4030 formulations were slightly higher (J = 1.4-3.5 $\cdot 10^{-8}$ g/(cm^2·s) than the reference Ciclopoli® (J = 1.1$\cdot 10^{-8}$ g/(cm^2·s). The permeation coefficient (P = flux/drug concentration) was 4.6 – 6.0 times higher than the one determined for Ciclopoli®. Moreover, microbiological studies using the infected nail plate model with the fungus Trichophyton rubrum showed an entire fungal growth inhibition by applying the poloxamer 407-based formulation 1P4030 onto KF and hoof plates (scores 0, n = 8). Due to a high water content of 30 % in the vehicle P4030, swelling of the nail bed and thus an increase in drug permeation is likely to occur. IPA content of 15 % may act as permeation enhancer. Furthermore, as a lipophilic drug of low molecular weight (207.27 g/mol) (3), CPX may be released quickly from a hydrophilic vehicle such as the poloxamer 407-based formulation.

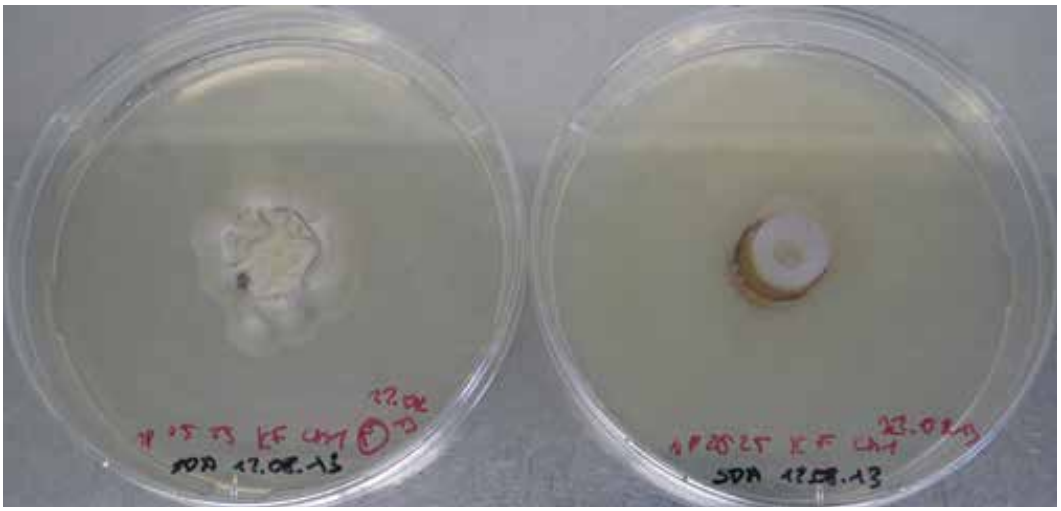

Fig. 1: A: positive control (KF) without treatment (score: 10); B: KF treated with 1P2525 (score: 0)

The vehicle P2525 had a cream-like appearance with a water content of 50 % and an IPA content of 12.5 %, respectively. A maximum of 4 % CPX was dissolved in the vehicle leading to a liquid and translucent macroscopical appearance. Permeation studies through a keratin film indicated a 2.9 – 5.4 times higher flux and an approximately 10 times higher permeation

coefficient in comparison to Ciclopoli®. Regarding the infected nail plate studies, the formulation 1P2525 showed an almost complete fungal inhibition on KF (score 0.125, n = 4) and on hoof plates (score 0.188, n = 8).

Conclusion: The present contribution shows the influence of the vehicle on the in vitro drug permeation. Poloxamer 407-based formulations with low drug content, but higher fluxes and permeation coefficients than the marketed nail lacquer Ciclopoli® were successfully developed. Moreover, microbiological studies showed that P4030 formulations with only 1 % ciclopirox olamine as API were as effective as Ciclopoli® and P2525 with 1 % API were slightly less effective than Ciclopoli® on KF.

In conclusion, we have developed poloxamer 407-based formulations with acceptable permeation behaviour and antifungal activity despite low drug content.

References:

[1] Baran, R.; Hay, R. J., Garduno, J. I. Review of antifungal therapy and the severity index for assessing onychomycosis: Part I. Journal of Dermatological Treatment 2008. 19 (2), 72-81.
[2] Lusiana; Reichl, S.; Müller-Goymann, C. C. Infected nail plate model made of human hair keratin for evaluating the efficacy of different topical antifungal formulations against Trichophyton rubrum in vitro. European Journal of Pharmaceutics and Biopharmaceutics 2013, 84 (3), 599-605.
[3] Hui, X.; Wester, R. C.; Barbadillo, S. et al. Ciclopirox delivery into the human nail plate. Journal of Pharmaceutical Sciences 2004, 93 (10), 2545-2548.

Wissenschaftliche Posterausstellung: Poster 20

PPAR agonists upregulate filaggrinm expression and improve lipid composition and organization in a filaggrin knock down skin model

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Loss-of-function mutations in the gene encoding for filaggrin (FLG) are the major predisposing factor for atopic dermatitis (AD). As for today, therapeutic options for FLG associated skin diseases only alleviate the symptoms such as dry and itchy skin or reduce the inflammation. No therapy exists preventing the development of these symptoms or restoring the disturbed skin barrier function. Peroxisome proliferator-activated receptor (PPAR) agonists are not only important therapeutics for the treatment of lipid disorders and diabetes but also exhibit beneficial effects in patients suffering from inflammatory skin diseases like AD. PPAR agonists are known to increase the expression of FLG in skin and positively influence the skin barrier homeostasis, skin barrier recovery and stratum corneum (SC) integrity (1). In order to study the effects caused by a lack of FLG in vitro we established a FLG knock down skin model (2, 3). In this study, we evaluated the effects of the PPAR agonists' docosahexaenoic acid (DHA) and clofibrate (CLF) in a FLG deficient (FLG-) skin model in terms of FLG expression, skin lipid organization and composition and skin permeability. We detected an about 18.96-fold upregulation of FLG in DHA treated normal skin models (FLG+). FLG expression increased significantly about 2.69-fold in FLG- models upon DHA treatment even exceeding the FLG expression of DHA untreated FLG+ samples. These results were confirmed on the protein level. Histological examination revealed a thickening of the SC upon DHA treatment (FLG-/DHA- 8 ± 1.2 µm vs. FLG-/DHA+ 12.8 ± 1.5 µm). In terms of skin lipid composition, a treatment with DHA normalized the pathologically increased free fatty acid (FFA) levels: FFA amounts were reduced from 22.0 ± 2.7 µg/mg to 15.3 ± 1.0 µg/mg in FLG- models following DHA treatment (FLG+ 13.3 ± 1.5 µg/mg). Furthermore, the skin lipid organization was significantly improved in FLG- constructs as determined by ATR-FTIR. Interestingly, skin absorption studies did not show an improvement of the skin barrier after DHA treatment. The beneficial effects on the skin barrier homeostasis are undoubted but further studies are necessary to completely understand the effects of PPAR agonists on the skin barrier function.

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References

1. Michalik L, Wahli W (2007) Peroxisome proliferator-activated receptors (PPARs) in skin health, repair and disease.
Biochimica et biophysica acta 1771:991-998
2. Küchler S, Henkes D, Eckl KM, Ackermann K, Plendl J, Korting HC, et al. (2011) Hallmarks of atopic skin mimicked in
vitro by means of a skin disease model based on FLG knock-down. Alternatives to laboratory animals: ATLA 39:471-480
3. Vávrová K, Henkes D, Strüver K, Sochorova M, Skolova B, Witting M, et al. (2013) Filaggrin Deficiency Leads to Impaired Lipid Profile and Altered Acidification Pathways in a 3 D Skin Construct. J Invest Dermatol.

Wissenschaftliche Posterausstellung: Poster 21

Organotypic Models mimic Premature Ageing and Cutaneous Squamous Cell Carcinoma

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Abstract:
The failure of investigational new drugs in clinical trials (95% for all indications [1] emphasizes the need for relevant human-cell based approaches. This can be due to the low predictive value of animal-generated pharmacological data for humans [2]. Underestimation of age-related changes might be of relevance, too. Considering skin, ultraviolet (UV) irradiation causes the most prevalent skin disorders in later phases of the life span, skin aging and cutaneous squamous cell carcinoma [3]. However, current drug development relies on (animal-based) disease models using juvenile rodents. In this study, we aimed to transfer the effect of UV irradiation to the lab scale by introducing organotypic models of premature ageing and cutaneous squamous cell carcinoma.

To induce premature ageing [4], we reconstructed epidermis from irradiated juvenile normal human keratinocytes with UV-B light (9.6 to 30 mJ/cm^2; single-dose). UV irradiation causes elevated ß-galactosidase expression in keratinocytes. The model showed irregular structure of stratum corneum and stratum granulosum with altered expression of epidermal differentiation markers (keratin-10, -14, involucrin, and filaggrin) and elevated IL-8-expression. Normal reconstructed human epidermis was built from non-irradiated keratinocytes.

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stratum corneum and stratum granulosum with altered expression of epidermal differentiation markers (keratin-10, -14, involucrin, and filaggrin) and elevated IL-8-expression. Normal reconstructed human epidermis was built from non-irradiated keratinocytes.

To mimic cutaneous squamous cell carcinoma we co-cultured human squamous cell carcinoma cells (SCC-12) with juvenile normal human keratinocytes on a dermal equivalent consisting of normal human dermal fibroblasts [5]. Normal reconstructed human skin was built without SCC-12 cells. Alteration of culture conditions allows controlling the tumor stage from carcinoma in situ to the invasive disease. Tumor nests show malignant histology and increased proliferation (Ki-67).

In conclusion, disease models on the cutting edge of tissue engineering offer test platforms for investigational new drugs. Further studies will focus on the absorption and metabolism profiles of our disease models.

References

[1] Hartung et al.: ALTEX. 2013. 30: 275-291.
[2] Tralau et al.: Environ Health Perspect. 2012; 120: 1489-1494.
[3] Stockfleth et al.: Eur J Dermatol. 2008,18: 651-659.
[4] Lewis et al.: Mol Biol Cell. 2008, 19: 1346-1353.
[5] Ali-von Laue et al.: Skin Pharmacol Physiol. 2014, 27: 173-180