Abstracts

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Wissenschaftliche Posterausstellung 2015: Poster 1

Individual analysis of epidermal barrier lipids and their composition in subjects with atopic dermatitis. Investigations using Lipbarvis®, a non-invasive sampling technique, and subsequent electron microscopic (LBV TEM®) and HPTLC analysis (LBV Lip®)

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Lipbarvis® is a new, innovative analysis method for the study of the epidermal skin barrier. Using a special glue-carrier system, non-invasive skin samples are taken, analyzed and mapped in transmission-electron microscopy (TEM). Specialized software determines the length of the lipid lamellae in relation to the intercellular space. The number of lipid lamellae permits very accurate conclusions about the effectiveness of the applied drug. This is followed by a quantitative evaluation of the individual samples and thus an exact description of the skin barrier. The skin lipids from the collected samples are separated by HPTLC. A distinction is made between the lipids cholesterol, free fatty acids and the ceramides EOS, NP and NH. A densitometry analysis is used to quantify the lipids.

The results presented here show that the lipids of the epidermal barrier in subjects with atopic dermatitis can be divided into different subclasses in terms of the respective lipid ratios. For some subjects, the ratio of cholesterol to ceramide EOS reversed nearly completely for very dry skin and atopic dermatitis. However, other subjects showed different lipid ratios among each other. And the ratios of fatty acids to ceramides also changed.

Individual examples are used to demonstrate that there is a correlation between biophysical parameters and the electron microscopic analysis, as well as the changes in barrier lipids found in the individual analysis before and after the treatment.

Wissenschaftliche Posterausstellung 2015: Poster 2

SKIN DISC ANTIBIOGRAM: A NEW IN-VITRO MODEL TO TEST PHARMACO-DYNAMIC EQUIVALENCE OF ANTI-BACTERIAL TOPIC FORMULATIONS

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INTRODUCTION

Generics represent an important part of new products on market. However there is a necessity to prove the equivalence between originator and generic products to obtain marketing authorization. The World Health Organization defines two products as therapeutically equivalent if they are pharmaceutically equivalent at the same dose and demonstrate similar safety and efficacy based on bioequivalence, pharmacodynamics, clinical or in-vitro studies; therapeutic equivalence is then assumed without requiring further supportive evidence (1).

Concerning antimicrobial products which target microbes rather than humans, supplementary appropriated tests should be used to establish efficiency against microorganisms (2).

The aim of this study was to adapt a well-known and described in-vitro assay “antibiogram disc diffusion” to prove and compare the efficiency of originator and generic antibiotic ointments. The investigated topical formulations are used for treatment of localized skin infections.

The Kirby-Bauer method (3) also called “disc diffusion antibiotic sensitivity testing” is primarily used by the clinicians to assess local susceptibility rates, as an aid in selecting empiric antibiotic therapy, and in monitoring resistance trends over.

In this method, small discs containing different antibiotics, or impregnated paper discs, are dropped in different zones of the culture on an agar plate, which is a nutrient-rich environment in where bacteria can grow. The antibiotic will diffuse in the area surrounding each tablet, and a disc of bacterial lysis will become visible. The diameter of the inhibition zone is proportional to the sensitivity of the microorganism and the efficacy of the antibiotic.

In the described method, human dermatomed skin discs containing the antibacterial formulation on the stratum corneum were used instead of antibiotic impregnated discs, to evaluate the equivalence of products (generic and originator).

MATERIALS AND METHODS

In a pre-experiment the skin permeation behavior of antibiotic from the two formulations was

evaluated. The experiment was performed in sixfold with one skin donor (max. 300 µm mean thickness).
The permeated amount of the active ingredients was quantified over a time period of 48 hours by withdrawing 8 samples from the acceptor compartment of Franz diffusion cells.

The Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) values were determined for both products on Staphylococcus aureus using broth microdilution method (4). Samples over MIC point were plated on Muller Hinton Agar, incubated 24h at 37°C and colonies counted to determine MBC values.

In order to evaluate if a brief skin disinfection using ethanol at 70 % or the skin itself can have any influence on the bacterial growth, previously sterilized dermatomized (max. 300 ?m mean thickness) human skin discs were prepared in 13 mm diameter and incubated on agar plates previously inoculated with the reference pathogen, Staphylococcus aureus. The survival of the microorganism was evaluated on different incubation times (18, 24 and 48 h).

After development of the method, bacteriostatic activity of generic and originator ointments were investigated and compared. The experiment was performed in sixfold.

RESULTS AND DISCUSSION

Results for both originator and generic formulations tested on Staphylococcus aureus are respectively MIC= 0.051 and 0.050 $\mu g \cdot mL^{-1}$, MBC= 3.256 and 3.212 $\mu g \cdot mL^{-1}$. The MIC and MBC values for both products are similar.

When comparing the ointments bacteriostatic activity using the “Skin disc antibiogram” no difference was observed regarding the inhibition zones between the originator and the generic (2.0 ± 0.2 cm versus 1.9 ± 0.2 cm). The experiment was performed in sixfold and confirmed the reproducibility of the results. Furthermore, no inhibition was observed after incubation in the control plates containing only skin without formulation. Even if the skin was only briefly and gently disinfected with 70 % ethanol, this procedure was sufficient to avoid a cross contamination of tested microorganisms.

Results are in accordance with permeation measurements: originator presents a Papp value of 8.39E-10 ± 7.43E-11 $cm \cdot s^{-1}$ and a cumulative transport of 1.74 ± 0.44 $\mu g \cdot cm^{-2}$ while the generic Papp value is 1.11E-09 ± 2.68E-10 $cm \cdot s^{-1}$ and the total permeated amount of API after 48 h 2.66 ± 0.48 $\mu g \cdot cm^{-2}$. The transport and the absorbed amount of API are similar for both formulations.

From this assay the generic and originator can be considered as equivalent.

CONCLUSION

The described method is the basis of a new tool as ex vivo pharmacodynamics equivalence test, using the principle of agar plate diffusion antibiogram. The application fields may be “in-vitro bioequivalence” testing of generic products, but also the galenic development of innovative antibacterial formulations.

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Wissenschaftliche Posterausstellung 2015: Poster 3

Anti-proliferative and anti-migratory effect of hyperforin on human dermal fibroblasts

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Introduction:
Hyperforin (HYP), a bicyclic polyprenylated phloroglucinol derivative, is one of the main active ingredients of Hypericum perforatum [1]. Apart from its known antidepressant activity, other effects such as anti-inflammatory, anti-microbial, anti-proliferative and differentiation-inducing action were identified after in vitro application of HYP on keratinocytes [2]. Therefore, HYP was suggested as an interesting candidate in topical therapy of skin diseases such as psoriasis, atopic dermatitis or fibrosis. In the present study HYP was used as stable hyperforin dicyclohexylammonium salt (HYP-DCHA). The influence of different HYP-DCHA concentrations on primary human dermal fibroblasts (HDF) isolated from neonatal foreskin was investigated with different assays in order to substantiate the potency of HYP and to establish an experimental in vitro setup for prospective investigations of HYP-DCHA formulations.

Methods:
HDF were cultivated in DMEM supplemented with 10 % fetal calf serum (FCS), 2 mM l-glutamine and antibiotics under standardized culture conditions. HYP-DCHA was dissolved in DMSO, subsequently diluted with medium to different concentrations with a final DMSO concentration of 0.1% (v/v). Viability of HDF was performed with an MTT assay. Additionally a cytotoxicity assay (CellTox™ Green cytotoxicity assay, Promega) was performed as well as a life-dead staining with fluorescent dyes, i.e., Hoechst 33342, calcein and propidium iodide. For all these assays the cells were incubated for 24 h along with the different HYP-DCHA concentrations.

Investigation of a potential anti-proliferative effect was done by determining the cell number of HYP-treated cells once a day over a time period of 4 days with a Z2 Coulter-Counter system (Beckman Coulter, Krefeld, Germany).

A scratch assay was performed in order to analyze the impact of HYP-DCHA on the migration behavior of treated cells. Micrographs of the scratch were taken with a confocal laser scanning microscope Nikon eclipse Pi (Nikon, Tokyo, Japan) half-hourly within at least 16.5 h.

Results:
The MTT test showed no significant loss of viability upon HYP-DCHA incubation at concentrations of 50 nM to 1 µM. At 5 and 10 µM HYP-DCHA, a slight decrease in viability was

detected, whereas the cytotoxicity assay did not display any cytotoxicity at any tested HYP-DCHA concentration. The latter was confirmed by life-dead staining which did not show any cytotoxicity of HYP-DCHA. The cell counting experiment clearly showed a dose-dependent anti-proliferative effect of HYP-DCHA on HDF cells. That means in the present study, the decrease in viability is not an indication of cytotoxycity but with regard to the reduce cell number a strong hint at an anti-proliferation effect. In Addition the scratch assay clearly showed an anti-migratory effect of HYP-DCHA-treated HDF cells at a 10 µM HYP-DCHA concentration.

Conclusion:
HYP-DCHA does not only affect human epidermal cells as previously described in literature but also inhibits proliferation and migration of HDF cells.

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Wissenschaftliche Posterausstellung 2015: Poster 4

smartPearlsTM – novel dermal delivery system for amorphous cosmetic and pharma activess

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Achieving a sufficient penetration and bioactivity of poorly soluble actives is a challenge in formulation technology. A simple but very efficient approach is the increase in saturation solubility Cs, thus increasing the concentration gradient Cs-Cskin between formulation and skin, and subsequently the diffusional pressure. This can be achieved by transforming the active powder into the nanodimension, i.e. producing nanocrystals [1] (e.g. smartCrystals®). Alternatively the active can be transferred from the crystalline to the amorphous state, being even more efficient – but the amorphous state has physical stability problems excluding the dermal use. Ideal would be to combine the solubility enhancing effects of size reduction and amorphous state – this was realized in the smartPearlsTM delivery system [2].

The delivery principle was previously employed for oral delivery [3] (CapsMorph®), and has now been transferred to dermal delivery. The cosmetic or pharma active is caged in the pores (typically 2-100 nm) of meso- or macroporous materials, e.g. silica (Syloid 3D, company Grace). The space restriction prevents re-crystallization, the amorphous state was proven stable up to 5 years [4]. The silica particles are loaded by the impregnation method or spray-drying, and the loaded silica particles simply dispersed in the water phase of gels or creams.

The anti-oxidants rutin and hesperdin as model actives were loaded onto Syloid® SP53D-11920 (SYLOID® 3D). These smartPearlsTM were further studied in a porcine ear skin test to investigate the penetration behavior. The loading of active was 32.0% by using the wetness impregnation method. The amorphous state was verified for 6 months (until now) by x-ray diffraction (XRD). smartPearlsTM were incorporated into a 5% hydroxypropyl cellulose (HPC) gel. The dermal formulations were physically stable by judging from microscopy (absence of silica particle aggregations) and XRD (no crystal peaks appeared).

smartPearlsTM gels with only 1% active were applied to the pig ear tape stripping. Controls were 5% raw drug powder (RDP) gels and 5% nanocrystal (NC) gels. In absolute terms, the smartPearlsTM formulation were slightly superior to nanocrystals, the difference became very clear after normalization of the data to 1% active content. Results were “normalized” dividing the drug amount (µg) per strip by the active concentration (%) in the applied formulation. Both

smartPearlsTM formulations showed clear superiority.

The smartPearlsTM technology stabilizes efficiently the amorphous state in porous materials, shows similar or even better dermal penetration than nanocrystals, is industrially feasible, and thus a promising dermal delivery technology for poorly soluble actives. The particle size of the smartPearls is typically 10-40 µm, thus outside the nano size range and no “nanoparticle” product. This is of increasing importance for the consumer due to the nanotoxicology discussions.

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Wissenschaftliche Posterausstellung 2015: Poster 5

Investigation of an antioxidant containing ointment as therapeutic and preventive strategy for hand foot syndrome in cancer patients

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Background: Dermal side effects of chemotherapy such as the hand foot syndrome (HFS) can cause a major impairment in the quality of life of oncologic patients. Notably high incidences of up to 78% of HFS can be found in patients treated with pegylated liposomal doxorubicine, 5-fluorouracil, docetaxel and paclitaxel. The symptoms of HFS are classified into 3 severity grades, ranging from light erythema, edema and numbness to painful blisters, rhagades and erosions causing severe pain in affected patients. Up to now no preventive or therapeutic option has been found to effectively avoid HFS in order to continue chemotherapy. Hence, dose reduction or discontinuation of chemotherapy are frequent measures in HFS grade 3 patients. Here, radical formation after inverse penetration of chemotherapeutics in the skin as possible pathomechanism of HFS was shown and the effectiveness of a therapeutic and preventive strategy investigated based on these findings.

Methods: The visualization of the inside-out penetration of chemotherapeutics on the skin surface of patients treated with pegylated liposomal doxorubicin (PLD), 5-fluorouracil (5-FU), docetaxel (DT) and paclitaxel (PT) was investigated using laser scanning microscopy (LSM) in order to determine a possible local toxic effect.

Since such a toxic effect could likely be caused by radical formation, the carotenoid concentration of the skin was measured in these patients using resonance Raman spectroscopy. Measurements were conducted right before and after systemic application of chemotherapy in order to determine changes in the antioxidant status caused by radical formation.

Furthermore based on these previous investigations, a preventive strategy was designed using an antioxidant containing ointment applied in 17 patients receiving chemotherapy.

Results: It was shown that PLD was the only detectable chemotherapeutic on the skin surface by LSM after systemic application due to its fluorescent properties. A strong fluorescent signal was detected in the sweat glands, spreading over the skin surface.

The antioxidant status showed a decrease after application of all investigated chemotherapeutics. While 5-FU and PT showed only a moderate change in antioxidants, the most prominent decrease was found in PLD patients.

Since these findings suggested a local toxic effect of PLD in the skin, the preventive application of an ointment with a high radical protection factor was investigated in PLD patients and compared to a placebo ointment. Here, PLD showed a significant effect in the prevention of PLD-induced HFS grade 3 ($p=0.003$, Fisher's exact test), a lower overall incidence of PPE and significantly lower grades ($p=0.026$) of HFS compared to placebo. Furthermore patients, who had developed a PPE grade 3 were immediately treated with the antioxidant ointment due to ethical reasons and showed an alleviation of skin symptoms so that chemotherapy could be continued as planned in these patients.

Discussion: An ointment with a high radical protection factor can be an effective preventive and therapeutic option for HFS accompanied by an important improvement of quality of life in affected patients. Further and larger studies will be necessary to confirm the effectiveness of this approach.

Wissenschaftliche Posterausstellung 2015: Poster 6

Stability and skin compatibility of an adapalene-loaded solid lipid microparticle dispersion

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Introduction: A novel adapalene-loaded solid lipid microparticle dispersion consisting of 13.93% hydrogenated palm oil, 5.97% lecithin, 0.1% adapalene, 12% poloxamer 407, 3% polyethylene glycol 12000, 0.2% potassium sorbate, 0.1% citric acid, and 64.7% water (all by weight) exhibits potential for follicular penetration and targeted drug delivery and release in sebum [1,2]. In the present study, the stability regarding particle size, melting behavior, and drug content at different storage temperatures of 4°C, 23°C, and 40°C was monitored for 4 weeks. Furthermore, skin compatibility was evaluated by an MTT test in comparison to the commercial cream formulation Differin® after incubation of various dilutions in Dulbecco's Modified Eagle Medium (supplemented with 10% fetal calf serum, 2 mM l-glutamine and antibiotics) on a HaCaT monolayer under standardized culture conditions.

Results: The particle size determination revealed an increase of the mean particle size from about 3.7 to 4.2 µm after 4 weeks of storage at 23°C, whereas the size slightly decreased to about 3.5 µm at 4°C and increased significantly to 4.7 µm at 40°C. Higher storage temperatures may promote an Ostwald ripening. The particle size distribution displayed a second mode in the nanometer range after storage at 40°C potentially due to the preferential leakage of lecithin into the aqueous phase. At 23°C and 40°C, a slight increase of the melting temperature from approximately 55°C to 56°C and 57°C, respectively, was detected, whereas the initial melting at 55°C remained nearly constant during storage at 4°C. The drug content of 0.1% was stable and the pH value remained in the range of about 5.4 for 4 weeks.

The MTT test showed a cell viability of about 80-90% referred to medium control for the 1:10, 1:100, and 1:1000 dilutions of the novel lipid particulate dispersion. In contrast to that, the cell viability of the HaCaT monolayer decreased to about 11% in the case of the 1:10 dilution of the Differin® cream. The less concentrated dilutions of 1:100 and 1:1000 still featured lower cell viabilities of 27% and 69%, respectively. This significant difference might be caused by crucial constituents of the Differin® cream such as polyethylene glycol methyl glucose sesquistearate since cell toxicity on HaCaTs depends on the type of the surfactant [3]. Methyl paraben may also decrease the cell viability [4]. On the other hand, hard fat and lecithin as components of the solid lipid microparticle dispersion do not negatively affect HaCaT cells [5].

Conclusion: The chemical stability of adapalene is independent of the storage temperature of the formulation while the physical stability of the formulation is high at 4°C and limited at 40°C. Furthermore, the novel formulation is milder to HaCaT cells and may feature less irrita-

tion potential than the cream. The latter might be a benefit in topical retinoid therapy.

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Wissenschaftliche Posterausstellung 2015: Poster 7

Betulin formulated as smartCrystals® for increasing dermal bioavailability

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Betulin, a bioactive substance extracted from the outer bark of Betula pendula, has been used as an active ingredient for the treatment/support of treatment of various skin diseases, such as eczema and psoriasis [1]. Current commercial products with betulin are Pickering emulsions. However, its poor solubility both in water and lipophilic media limits its bioavailability and absorption in the skin. To circumvent this problem, betulin was produced using the smartCrystal®-technology (nanocrystals), which can increase skin penetration by 3 different effects:
a) increased saturation solubility und thus increased concentration gradient between formulation and skin,
b) increased dissolution velocity and c) high adhesion to the skin.

The penetration was investigated in vitro by the pig ear tape strip test. Additionally, the physical stability of betulin smartCrystals® was analyzed over one year.

The production of Betulin smartCrystal® suspension was performed by two methods: a) high pressure homogenization (HPH) applying 20 cycles at a pressure of 1500 bar; b) bead milling (BM) using 0.1 mm milling beads and a milling time up to 30 minutes. Five different surfactants were investigated: Plantacare 810 UP, Plantacare 818 UP, Plantacare 1200 UP, Plantacare 2000 UP and tocopheryl polyethylene glycol succinate (TPGS). 2% drug was dispersed in 1% surfactant solution by Ultra-Turrax for 10 seconds at 8000 rpm. Subsequently, the resulting raw suspensions were applied to the two production methods. The physical stability of the nanosuspensions was evaluated by analyzing betulin samples which were stored at three different temperatures (4°C, 25°C, 40°C) for four different periods of time (0 day, 7 days, 1 month, 1 year). Mean particle size was analyzed by photon correlation spectroscopy (PCS) and zeta potential (ZP) was determined by electrophoretic light scattering. Possible larger crystals were measured by laser diffractometry (LD). Tape stripping (n=3) was performed with fresh pig ear skin and different betulin formulations were applied to the skin for 20 minutes. Drug concentrations in the strips were measured by HPLC.

Betulin smartCrystals® were successfully produced by HPH while bead milling failed. For all formulations, independent on the surfactant, the diameters of betulin smartCrystals® were below 400 nm (z-average). The four types of Plantacare were superior to TPGS in both production process and storage. When samples were stabilized with Plantacare, a diameter of around 300 nm was obtained and remained stable after one year of storage. Among these

various surfactants, Plantacare 2000 UP appeared to be the most efficient stabilizer, especially in the long-term storage. The ZP in water was -56.9 mV, indicating a good stability of these betulin smartCrystals®. Furthermore, the LD and PCS diameters showed limited increase when the samples were stored at 4°C and 40°C, proving physical stability of the nanosuspensions at different temperatures. According to the pig ear study, half the concentration of betulin smartCrystals® (1.8%) incooperated into a gel (5% hydroxypropyl cellulose) showed higher skin penetration compared to a commercial product with 3.7% betulin (e.g. drug amount in the 9th strip was 16.1 µg for smartCrystals® and 11.1 µg for the commercial product).

In summary, betulin was produced successfully as smartCrystals® by HPH as superior method compared to bead milling. The well tolerated Plantacare 2000 UP was found to be the most suitable surfactant both in production process and 1 year long-term stability. The 270 nm betulin smartCrystals® showed at only half the concentration (1.8%) a higher skin penetration than a commercial reference product. The data open the perspective to produce a more efficient dermal product.

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Wissenschaftliche Posterausstellung 2015: Poster 8

The integrity of dermal therapeutic DNAzymes in chitosan polyplexes

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DNAzymes represent a potential new class of nucleic acid-based active pharmaceutical ingredients (API). DNAzymes have the ability to regulate pathological gene expression on a post-transcriptional level by specifically binding to the targeted mRNA through complementary base paring. The bound target is cleaved by the catalytic domain of DNAzymes, resulting in an inhibition of the specific translation [1]. The combination of such specificity and enzymatic activity makes DNAzymes an interesting candidate for the therapy of different diseases.

Some potential DNAzymes are currently being tested for the therapy of skin diseases, including atopic dermatitis, psoriasis and actinic keratosis [2]. To achieve a successful topical treatment, a certain concentration of intact DNAzymes is necessary at the side of action. Therefore, the DNAzymes must be able to penetrate into the first layers of the skin while maintaining their integrity.

Especially during dermal application, DNAzymes are exposed to degrading enzymes. These enzymes are secreted ubiquitously by the skin and resident bacteria on the skin surface. The degradation of potential API reduces the concentration at the site of action and compromises the therapeutic outcome. To counteract degradation, we examined different protective systems for DNAzymes. The most promising approach was the complexation of DNAzymes with chitosan. In this self-assembly process, two polyelectrolytes complexed into polyplexes via electrostatic interaction.

The data of our studies confirmed that the particular DNAzyme could complex to polyplexes. The generation of polyplexes was controlled by the ratio of chitosan's free amino group to DNAzyme's phosphate (N/P ratio). The complexation of DNAzyme consequently reduced the abundance of free DNAzyme. A minimal recovery of less than 3.2 ± 0.2 % of free DNAzyme was achieved. The polyplexes could be decomplexed into basic components by adding hydroxide to the system. The intact DNAzyme could be completely recovered, indicating that the polyplexes did not affect the integrity of the DNAzyme. To validate the protective efficiency of polyplexes, the DNAzyme-chitosan polyplexes were incubated with a degrading deoxyribonuclease. Without protection, and at low N/P ratios, the DNAzyme was nearly completely degraded, while raising the N/P ratio achieved the maximal protective efficiency in our study. In conclusion, polyplexes represent a promising protective system for dermal application of therapeutic DNAzymes.

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Wissenschaftliche Posterausstellung 2015: Poster 9

Next generation after SLN® and NLC® – the “chaotic” smartLipids®

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In 1991 the solid lipid nanoparticles (SLN®) [1] were developed as alternative to liposomes, nanoemulsions and polymeric nanoparticles, in 1999 the second generation followed, the so called nanostructured lipid carriers (NLC®) [2, 3]. Now in 2014 the third generation was developed, the smartLipids® [4], possessing advantages compared to the first 2 generations.

All these lipid particles possess a lipidic particle matrix being solid at body temperature. In the SLN this matrix consists of a solid lipid, typically a single solid lipid. Disadvantage was limitation in drug loading due to the formation of highly ordered ? modification. Especially during storage a less ordered SLN particle matrix structure could re-order with time, i.e. forming increasing fraction of ? modification leading to drug expulsion and formation of drug crystals outside the SLN particle (e.g. in the water phase of gels and creams). Consequently in the next generation NLC a nanostructuring of the particle matrix was the aim, i.e. by mixing chemically (spatially) very different lipids, typically 1 solid lipid and 1 liquid lipid (oil). This created more imperfections in the lipid matrix, increasing drug loading and reducing drug expulsion during storage. However, in most NLC still a polymorphic transition occurred during storage.

Consequently the third generation of smarter lipids was developed. In these smartLipids® a “chaos” in the particle matrix is generated by blending about 10 different solid or solid and liquid lipids. Such a chaotic mixture is not able to form a densely packed structure any more, the particle matrix possesses a large or dominant fraction of ? and ?′modifications, no or limited ? modification. These particles possess the following advantages [5] compared to SLN® and NLC®:

- increased drug loading (e.g. 1% vitamin A in SLN®, 5% in NLC® and >15% in smartLipids®),
- no or little effect of surfactant on matrix structure, i.e. higher flexibility in choosing surfactants,
- no (or very limited) polymorphic transitions during storage and thus
- firmer drug inclusion on storage, no drug expulsion, long shelf life.

Based on this, the smartLipids® possess clear advantages for producing dermal formulations in cosmetics and pharma, but also for e.g. oral delivery. However, not each randomly taken mixture creates this chaotic structure, still a lipid selection based on certain structural

principles needs to be performed, followed by a screening to confirm the optimal %age of each lipid.

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Wissenschaftliche Posterausstellung 2015: Poster 10

Rutin smartCrystals® for an improved antioxidant activity in skin

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Rutin, as a naturally occurring flavonoid, shows promising high antioxidant activity on cellular level. Nonetheless, it cannot be used efficiently for dermal purpose due to its poor solubility in both aqueous and organic media. Since only dissolved active is able to create a concentration gradient and diffuse into the skin, the bioavailability is consequently insufficient. To circumvent this problem, the solubility of rutin has to be increased significantly. Thus, in present study, rutin was formulated using the smartCrystal®-technology and the penetration and antioxidant activity were compared to µm-sized raw drug powder.

Rutin smartCrystal® suspension was kindly provided by PharmaSol GmbH. As reference, a rutin raw drug powder (RDP) suspension was prepared with identical composition. The particle size distribution was analysed by laser diffraction (LD, Mastersizer 2000, Malvern Instruments, UK). A DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was carried out to compare the antioxidant activity of rutin smartCrystals® in intense lifting eye serum (Dr. JK Cosmeceuticals, Germany) with 8 other marketed anti-aging products, having rutin or its derivates incorporated as active agent. A methanolic extract of samples were added to a methanolic DPPH solution and the discoloration was investigated using a PharmaSpec UV-1700 photometer (Shimadzu Corporation, Japan). In addition, a tape stripping test was performed to compare the penetration strength and depth of topically applied rutin as smartCrystal® and µm-sized raw drug powder.

The rutin smartCrystals® showed LD diameters 50 % of 240 nm and 90 % of 860 nm, thus being in the nanosize range. DPPH study confirms the superior antioxidant activity of the rutin smartCrystals® in intense lifting eye serum (Dr. JK Cosmeceuticals GmbH, Germany). While all other tested anti-aging products are able to discolor at maximum 60 % of the DPPH solution, the intense lifting eye serum was able to decolorize more than 85 % within the same reaction time. In general, the tested products can be divided into 3 different antioxidant activity classes, from very strong discoloration of test solution (class I, > 80 %) equivalent to a very high antioxidant activity, to medium (class II, 60 – 20 %) and low discoloration of test solution (< 10 %) also standing for low antioxidant activity. The absolute and relative amount of the penetrated rutin from smartCrystal® hydrogel and RDP were compared among each other. In the upper layers of the stratum corneum (until the 7th tape strip) 1.5 fold higher amount of active could be found if applied as RDP. But in the more relevant deeper layers the penetration behaves inversely. Comparing the tape number 14 and 23 shows 2.2 and 2.5 times higher drug

amount for the rutin as smartCrystal®.

The antioxidant activity of intense lifting eye serum (Dr. JK Cosmeceuticals GmbH, Germany) containing rutin smartCrystals® proved to be superior in comparison to marketed products containing rutin formulated in standard manner. Rutin smartCrystals® show higher antioxidant activity combined with an increased skin penetration leading to an improved dermal bioactivity.

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Development of minoxidil nanocrystals for follicular delivery

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Drugs formulated as nanocrystals exhibit special features such as increased saturation solubility and dissolution velocity, resulting in increased bioavailability. For dermal application, yet another feature can be exploited: the fact that particles with a size bellow 700 nm have the potential of follicular targeting [1-3]. In the case of topical application of minoxidil, a nanocrystal formulation would not only allow targeting to the follicle but also create an alternative to the currently marketed formulations containing a high concentratrion of ethanol and/or propilene glycol. Therefore, in this study, the development of minoxidil nanocrystals for topical application was investigated.

Minoxidil powder (donation from Flamma S.p.A., Italy) was dispersed in various 1% stabilizer solutions, e.g. TPGS, Plantacare 2000 UP, Poloxamer 407 and Plantacare 810 UP, and processed by high pressure homogenization (HPH) using an LAB 40 (APV Deutschland GmbH, Germany). Additionally, selected formulations were processed by bead milling using a PML-2 (Bühler AG, Switzerland). Zeta potential and particle size were assessed by laser Doppler anemometry and photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments, UK) and light microscopy (LM).

Minoxidil is being used topically to treat hair loss. Because it is poorly soluble in water, the formulations on the market are ethanolic solutions, which can be irritative to the scalp. Besides, after evaporation of ethanol, minoxidil might re-crystallize, limiting its dermal penetration. For the production of a minoxidil aqueous nanosuspension, two production methods were investigated: high pressure homogenization (HPH) and wet bead milling. The particle sizes achieved by HPH were all around 1,400 nm. The best result was obtained for the stabilizer Plantacare 2000 UP after 15 HPH cycles (1,428 nm). But this size is still not sufficiently small. When this same formulation was processed by bead milling, after only 5 minutes milling, the particle size was already 330 nm and polydispersity index was 0.18. Longer processing did not further reduce the particle size, on the contrary, the nanosuspension destabilized and aggregated. These results were all confirmed by light microscopy.

The industrially feasible wet bead milling proved to be the best production method. Sufficiently small minoxidil nananocrystals in aqueous medium for follicular targeting could be successfully produced. By adapting the milling time, different sizes between 1000 nm and 330 nm can be produced, allowing optimization of nanocrystal size for follicular targeting.

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Wissenschaftliche Posterausstellung 2015: Poster 12

Individualized topical formulations – an important and essential therapeutical tool

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Background: There are still unresolved therapeutical issues on the pharmaceutical product market, which can partially be resolved by using standard prescriptions for formulations. Due to the new pharmacy practice order in 2012, magistral formula should be given the advantage over individual formulations due to the latter not being able to overcome the plausibility check of pharmacists. This causes communication problems between pharmacists and physicians. We evaluated the prescription behaviors of physicians in the topic "topical formulations" since 2011 – to evaluate the need for individualized prescriptions.

Methods: The analyzed data was compiled by the German Institute for Drug Use Evaluation (GIDE/DAPI). It contains information on the number of individualized prescriptions and the finished medicinal product. Data comparisons were made using different categories: region (Germany and the two individual districts Rheinland-Pfalz and Saarland), quarters (2011-2014), specialists (dermatologists, pediatricians, general practitioners), prescription profiles etc. The analyses were carried out with the SPSS-Software of IBM.

Results: Data includes information from 4th quarter of 2011 through the 3rd quarter of 2014. The data contains 1.912.964.745 prescriptions, including a volume of 92.634.644.947 €. 1.3% (25.619.489) are individualized formulations worth 509.529.621 €. The average cost of an individual prescription is 20€ to 21€ per package; for formulations prescribed in a consultations setting, the cost of one pack is between 11 and 12€. The cost of individualized compared to all prescribed drugs is 0,55%. 50% of dermatologists' prescriptions are finished medical products for topical treatment; among all prescriptions of topical treatments, 30% are prescribed by dermatologists. On average, a practicing dermatologist prescribed 245 individual formulations during the 3rd quarter of 2012.

Conclusions: Considering our findings, individualized formulations are necessary and helpful tools in treating patients with dermatological diseases. Further research should show which ingredients are used in those formulations and for which individual formulations there is an unmet need.

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IN VITRO PERMEATION AND PENETRATION OF CICLOPIROX OLAMINE FROM POLOXAMER 407-BASED FORMUALTIONS – COMPARISON OF ISOLATED HUMAN STRATUM CORNEUM, BOVINE HOOF PLATES AND KERATIN FILMS

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INTRODUCTION: Topical antifungal therapy of skin and/or nails is preferable in comparison to systemic therapy. However, it is accompanied by major challenges due to the low permeability of these tissues. Since fungal skin and/or nail infections (tinea pedis and onychomycosis, respectively) are common diseases, the objective of the present study was the development of dermal formulations targeting both. Therefore, the antifungal agent ciclopirox olamine (CPX) was incorporated into a variety of poloxamer 407-based formulations and analysed regarding its in vitro permeation and penetration performance across keratin films (KF) and bovine hoof plates as artificial nail models as well as across human stratum corneum (SC). The novel compositions consisted of poloxamer 407 (P407), double distilled water, propylene glycol (PG), isopropyl alcohol (IPA) and medium chain triglycerides (MCT) in given ratios.

EXPERIMENTAL METHODS: The P407-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). Subsequent storage was done for 24 h at 20 ± 1 °C to ensure sufficient equilibration. All the formulations were given codes reflecting their quantitative composition, e.g. 1P1050 represented a formulation loaded with 1 % CPX, while the vehicle itself contained 10 % P407/MCT (4:1), 50 % IPA/PG (1:1) and 40 % double distilled water (all w/w). Rheological measurements were performed with a HAAKE RheoStress 6000 rheometer (Thermo Fisher Scientific, Karlsruhe, Germany).

In vitro permeation studies (infinite dose technique) were carried out with modified Franz diffusion cells at 32 °C for 32 h. The receiver solution consisted of phosphate buffered saline (PBS) of pH 7.4. The quantification of the permeated and penetrated CPX amount was done

with high performance liquid chromatography (HPLC) (Waters, Eschborn, Germany). Moreover, infected nail plate studies were performed according to Lusiana et al.[1]

RESULTS: All analysed P407-based formulations exhibited semi-solid to liquid consistencies and were isotropic under a polarising microscope Leica DM LM (Leica Microsystems GmbH, Wetzlar, Germany). Upon CPX incorporation, the formulations became softer and the yield stresses decreased. Permeation coefficients from P407-based formulations across KF and bovine hoof plates and normalised retained CPX amounts in KF and bovine hoof plates were higher in comparison to a marketed nail lacquer as a reference. Data of KF and bovine hoof plates were comparable. With regard to SC permeation, the permeation coefficients were in the same range or higher compared to a semi-solid skin formulation, while the normalised retained CPX amounts in SC were higher in comparison with the reference. Infected nail plate studies with the dermatophyte fungus Trichophyton rubrum indicated complete growth inhibition on KF and bovine hoof plates for several P407-based formulations. On SC, 1P1050 completely inhibited fungal growth (score: 0), whereas the marketed semi-solid formulation did not show any inhibition after 6 days of incubation (score: 10).

CONCLUSION: P407-based formulations with a broad range of macroscopical appearances have successfully been developed being applicable to both skin and nail. In vitro permeation studies showed superior permeation for the P407-based formulations across the artificial nail models and equal to superior permeation across SC. Moreover, microbiological studies indicated complete fungal growth inhibition for several P407-based formulations. Data of KF and bovine hoof plates were comparable, so that KF are supposed to serve as an artificial nail model for in vitro permeation and infected nail plate studies besides the well-accepted model of bovine hoof plates.

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