Abstracts

Wissenschaftliche Posterausstellung

Gesellschaft für
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Wissenschaftliche Posterausstellung: Poster 1

Characterizing Beeswax-Jojoba Oil Nanosuspensions with TIO_2 as Inorganic Sunscreen with Emphasis on different Emulsifiers

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Organic and natural ingredients have become a major trend in cosmetics due to the consumers' wish for ingredients friendly to both the skin and the environment. This is especially true for sunscreens which play an important role in natural care cosmetics used for photo protection and thus skin cancer prevention.

A nanosuspension composed of titanium dioxide as inorganic sunscreen within a matrix of carnauba wax and decyl oleate in a 2:1 ratio has previously been reported to yield a high sun protection factor (SPF) of about 60 (in vitro). To render the formulations more interesting for natural cosmetics, ingredients were replaced by more eudermic ingredients with special regard to the emulsifiers.

Methods: Nanosuspensions were produced by dispersing a lipid phase into an aqueous phase using high-pressure homogenization. The sunscreen- loaded lipid nanoparticles were analyzed by particle size measurements (PIDS technique) and SPF analyses. Transmission electron microscopy (TEM) was then used to visualize the systems after freeze fracture of the nanosuspensions and negative staining with a 2 % [w/w] solution of uranyl acetate, respectively.

Results: The replacement of carnauba wax by bees wax not only showed a narrower particle size distribution but also a high SPF of about 80. The following replacement of decyl oleate by jojoba oil also showed a narrower particle size distribution and still a high SPF of about 75. In order to replace the polysorbate 80 (polyoxyethylene (20) sorbitan monooleate) used in the aforementioned formulation, the effect of emulsifiers qualified for natural care cosmetics (sodium lauryl sarcosinate, sucrose esters and potassium stearate each 1, 2 and 5 %) was investigated. The use of sodium lauryl sarcosinate and potassium stearate showed SPFs below 30 – a higher amount of emulsifier also yielded a lower SPF. The particle size distribution also became narrower with increasing percentage of emulsifier. Formulations containing 1 % of sucrose esterified with stearic acid, palmitic acid and lauric acid showed a very broad particle size distribution up to the micrometer range. SPFs of approximately 15 for sucrose stearate and palmitate were observed. Sucrose laurate showed an SPF of about 70. Due to the high SPF while using sucrose laurate, the amount of emulsifier was increased up to 5 and 10 %. These systems showed also high SPFs about 50 and 60- even more important, the particle size distribution improved considerably.

The morphology of the TiO2-loaded nanosuspensions was visualized after freeze-fracture and negative staining transmission electron micrographs and scanning electron micrographs. A close contact between bees wax and titanium dioxide crystals was confirmed. These systems look very promising and should be further investigated.

Wissenschaftliche Posterausstellung: Poster 2

Direct Evaluation of the Barrier Integrity by Visualization of the Intercellular Lipid Lamellae using TEM.Pilot Study with an Emollient on Children with Atopic Skin

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Treatment options of atopic dermatitis (AD) encompass the use of medicated and non-medicated preparations. One sign of atopic dermatitis is an impaired skin barrier which could be shown by either improved clinical features, by a reduced transepidermal water loss or by increased skin hydration. A direct evaluation of the barrier integrity is only possible by electron microscopical visualization and may be characterized by the lipid lamellar organization in the intercellular space between the corneocytes of the stratum corneum. Skin barrier integrity was measured by morphometric analysis of the stratum corneum inter-cellular lipid lamellae in non-lesional and eczemateousskin of atopic children and after a 15-day treatment (plus a 7 day follow-up) of atopic skin with a glycerin preparation. Furthermore, skin capacitance, transepidermal water loss and the local SCORAD were measured at the same time points.

A significant treatment effect was shown by the restoration of the inter-cellular lipid lamellae.This pilot study reveals that the morphometric analysis of the lipid lamellar organization is suitable to differentiate between healthy and diseased skin and to semi-quantitatively determine the effect of a non-medicated glycerin formulation. It is hypothesized that the technique is a promising tool to evaluate the strength of medicated and non-medicated preparations to normalized the skin barrier integrity.

Wissenschaftliche Posterausstellung: Poster 3

The molecular basis of the wound healing effects of betulins

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Delayed wound healing and chronic wounds are still severe problems in medicine today and a challenging task for the treating physicians. Skin-wound healing is a biological complex process divided into three phases: inflammation, new tissue building and tissue remodelling. Besides of the conventional remedies phytomedicines turned out to be an interesting alternative to beneficially influence these phases. Here extracts from birch bark (Betula pendula) have gained more and more interest.

Triterpenes from the betulin type are the active compounds of birch bark extract, which was recently shown to exert promising wound healing effects in patients [1]. Studies have been undertaken to explain these in vivo effects. We could demonstrate that birch bark extract and its main ingredient, betulin, influences the first phase of the wound healing process by increasing proinflammatory cytokines, chemokines and cyclooxygenase-2 (COX-2) in human primary keratinocytes. These mediators play crucial roles in cell migration, proliferation and angiogenesis. Consequently, deficiency of these mediators have been shown to remarkably impair wound healing [2,3,4]. We could provide evidence with COX-2 that its mRNA increase is due to a mRNA stabilizing effect.

Controlled migration of keratinocytes at the wound edge, which is necessary for reepithelialization, is a further crucial step in the wound healing process and requires a coordinated interaction of cytoskeletal elements. The reorganization of the actin cytoskeleton is thereby considered as an important driving force in cell migration [5]. We could demonstrate that lupeol had a strong effect on the actin cytoskeleton even in very low concentrations of 1 nM, which could be one explanation for the strong wound healing activity proven in the scratch assay. Studies are in progress to gain further insights in the complex wound healing mechanism of birch bark extract.

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Wissenschaftliche Posterausstellung: Poster 4

A novel in vivo model for the study of transdermal drug penetration

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Background: Transdermal drug delivery is a useful alternative pathway for many therapeutic agents. Thus, study and enhancement of the transdermal penetration is an important research goal. Our objective was to develop a novel experimental model which permits precise determination of the quantity of drug penetrating living full-thickness skin.
Materials and Methods: The experiments were performed on male SKH-1 hairless mice. A skin fold in the dorsal region was formed and fixed with two symmetrical fenestrated titanium plates. A circular wound was made on one side of the skin fold. A metal cylinder with phosphate buffer was fixed into the window of the titanium plate. A gel containing ibuprofen was applied to the other (non-wounded) side of the skin fold. The observation period lasted for 6h. Buffer samples were collected and blood samples were taken, as well. Concentration of the penetrated drug was measured by means of high-performance liquid chromatography. Further, microcirculation of the skin fold was observed with intravital videomicroscopy and histological analysis was performed, too.

Results: The skin fold was morphologically intact and had a healthy microcirculation. The drug appeared in the acceptor buffer after 30 min and its concentration exhibited a continuous increase. The presence of ibuprofen was also detected in the plasma.
Conclusion: Our model allows repeated measurements in the same animal, simultaneous studies of penetration and absorption and examinations of the microcirculation of the skin. This model may be a useful addition in the armamentarium of penetration studies.

Wissenschaftliche Posterausstellung: Poster 5

Influence of baby lotion on the skin barrier function of healthy infants after baby swimming

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Introduction
In order to characterize the skin barrier function that matures during the first year of life it is important to study external effects, such as skin care and contact with water. In this clinical study, we researched the effects of a baby lotion, applied to the skin after baby swimming, on the skin barrier function of infants.

Participants and Methods
44 healthy infants aged 3-6 months were randomized into two groups in this monocentric, prospective study: in groupL, baby lotion was applied on the entire body, and in groupWL, no lotion was used after baby swimming. Transepidermal water loss, stratum corneum hyrdation, skin-pH and sebum were measured on four body regions using non-invasive methods.

Results
In groupL, skin surface lipids and pH remained stable. In groupWL skin surface lipids decreased on the forehead and thigh and pH on the thigh and buttock. Fewer infants in groupL had at least one adverse event compared to groupWL. Differences in the skin barrier function between boys and girls were seen on the different body regions.

Conclusions
The skin barrier function reacted differently in regards to the body region on the care regime after baby swimming. Influence of baby lotion on skin barrier and gender differences in skin functional parameters were demonstrated for the first time in healthy infants participating in baby swimming.

Keywords: Skin barrier maturation, Infant, baby swimming; TEWL, pH, Sebum, Gender

Wissenschaftliche Posterausstellung: Poster 6

Delivery of hydro- and lipophilic antioxidants to the skin measured by in vivo Electron Paramagnetic Resonance (EPR) spectroscopy

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The intake of nutritional supplements such as vitamins and antioxidants is popular but still controversially discussed. Manufacturers of these supplements are advertising their products with efficient quenching of free radicals and, therefore, the reduction of premature skin ageing. However, the question arises as to whether these active ingredients are delivered to the skin and if they can unfold their beneficial properties in the skin.

Besides resonance Raman spectroscopy, which determines carotenoids non-invasively in the skin, EPR spectroscopy was utilized for the first time to determine the skin antioxidative capacity in vivo before and after the intake of nutritional supplements. The antioxidative capacity was determined by applying a reactive test radical to the skin, measuring the decline of the test radical and by the determination of the rate constant. Moreover, Raman spectroscopy was utilized for the determination of skin carotenoids. 55 volunteers (5 groups) were included in the study and randomly assigned one of the following products: Aronia energy, a natural chokeberry peel extract, containing mainly hydrophilic antioxidants, Lutex, a natural extract from curly kale, containing mainly lipophilic antioxidants (carotenoids), pure vitamin C and dextrose or sugar capsules as placebo.

The radical scavenging activity of the skin was enhanced after supplementation in all verum groups, the decrease in TEMPO was faster and the rate constant increased. No differences could be found in the placebo groups. The rate constant increased in all verum groups by approximately 23% after 4 weeks intake. An increase in skin carotenoids was only observed for lipophilic antioxidants from Lutex EPR spectroscopy is a versatile tool for the evaluation of beneficial effects of natural extracts. Besides the detection of topically applied antioxidants, EPR spectroscopy and the application of test radicals also allow the detection of systemically applied antioxidants.
n order to characterize the skin barrier function that matures during the first year of life it is important to study external effects, such as skin care and contact with water. In this clinical study, we researched the effects of a baby lotion, applied to the skin after baby swimming, on the skin barrier function of infants.

Wissenschaftliche Posterausstellung: Poster 7

Rheological method to develop novel cerates as potential substitutes for White Soft Paraffin

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Introduction

Due to the natural limitation of crude oil and new safety precautions for the paediatric population [1], there is the need for alternative ointment bases for the commonly used White Soft Paraffin (WSP).
Cerates, defined as a mixture of natural or semisynthetic wax and liquid oil, are promising candidates for substitution.
For non-Newtonian substances like WSP, there are two aspects of particular importance:

- They show reconstruction over time [2].
- Rheological parameters depend on the structure-generating compounds [3].

Aim

The aim of this work was to improve a rheological method [2] in order to characterize novel semi-solid substitutes for WSP and to facilitate pharmaceutical development of new medicines.

Material and methods

WSP (Caesar & Loretz, Hilden, Germany), was compared to cerates containing White Beeswax (WB) (Caesar & Loretz, Hilden, Germany) and Medium-Chain Triglycerides (MCT) (Miglyol 812, Sasol, Hamburg, Germany). WB was melted together with MCT at 80-85 °C on a water-bath and allowed to cool down to room temperature while stirring manually. The mixtures were prepared with different WB quantities, from 12,5 % to 22,5 %. WSP was treated in the same way.

The rheological measurements were performed with a Kinexus Rotational Rheometer (Malvern Instruments, Worcestershire, United Kingdom) with cone-plate equipment: 20 mm diameter with 1° cone-angle and a stainless steel plate.

Wissenschaftliche Posterausstellung: Poster 8

Effects of InlB321-CD in solution and hydrogel formulation on immortalized human keratinocyte cell line

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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 c-Met. It was proposed that activation via receptor dimerization is mediated through 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 c-Met receptor which controls amongst others proliferation and migration. After being stimulated by its endogenous agonist hepatocyte growth factor (HGF), which is secreted by e.g. dermal fibroblasts, this receptor plays an important role in the regeneration of the epidermis.

In previous studies, the mitogenic properties of InlB321-CD have already been tested on immortalized dermal keratinocytes (2) and premature organotypic co-culture (3). Furthermore, InlB321-CD stimulation was shown in a wound healing assay (3).

In order to distinguish migration from proliferation in the present study, an in vitro 'wound healing' assay of a confluent HaCaT monolayer was performed subsequent to mitomycin C treatment which inhibits mitosis. Additionally, the present project aims at incorporating InlB321-CD in a formulation which is intended to be tested on modified organotypic co-culture in upcoming tests. For this purpose, a hydrogel formulation e.g. a hydroxyethylcellulose gel (HEC gel) was chosen since hydrogels were found to trigger wound healing in vivo and in vitro (4) even without any active compound. Moreover, hydrogels might be proper vehicles for protein drugs. The stability of InlB321-CD in this formulation should be investigated with regard to maintaining its mitogenic activity. Therefore, a proliferation assay was carried out on immortalized dermal keratinocytes subsequent to incubation with InlB321-CD that was incorporated in HEC gel versus InlB321-CD in solution.

Methods:
A confluent HaCaT monolayer was serum-starved (24 h), incubated with 10 µg/ml mitomycin C (2 h) and afterwards scratched with a pipette tip. The cells were washed with PBS and then incubated for 24 h with 0.5 nM InlB321-CD, 1 nM InlB321, 0.5 nM HGF or just medium. The

cultivation with medium served as negative control whereas that with HGF served as positive control. Partial 'healing' of the scratch area was documented with micrographs and quantified with an imaging software system (Olympus DP soft, Olympus).

In terms of proliferation assay, a hydroxyethylcellulose gel (0.6 %) was loaded with 25 nM InlB321-CD. The same concentration in PBS served as control. Storage conditions were 4 – 8°C for 24 hours and seven days, respectively, to check for any incompatibilities. Afterwards, the buffered InlB321-CD solution, the gel with active compound, and the plain formulation were diluted 50-fold with serum-free medium. Subsequently, serum-starved HaCaT monolayers were treated with 0.5 nM InlB321-CD incorporated in gel as well as in solution versus medium and plain HEC gel. After 24 hours of incubation, the proliferation was measured with an MTT assay.

Results: Subsequent to incubation with mitomycin C and 24 h after scratching, the monolayer of HaCaT cell line, treated with 0.5 nMdimeric InlB321 showed a significant smaller gap compared to incubation with medium. The cells treated with 0.5 nM HGF as positive control reduced the 'wound' gap as well, whereas 1 nM monomeric InlB321 did not stimulate the HaCaT cells. This result shows that InlB321-CD also acts as motogenic agent on immortalized dermal keratinocytes, whereas monomeric InlB321 does not show any migratory activity.

The proliferation assay showed the same mitogenic properties of InlB321-CD incorporated in HEC gel compared to InlB321-CD in solution both after 24 h and seven days of storage of the formulations. Furthermore, proliferation was higher by factor 1.8 than with plain HEC gel and medium. Hence, it can be concluded that InlB321-CD activity is not negatively affected by the gelling agent hydroxyethylcellulose.

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Wissenschaftliche Posterausstellung: Poster 9

Physiochemical Characterization of Topical Formulations containing NSAIDs (with Emphasis on Ibuprofen): Thermal Gravimetric Analysis and Differential Scanning Calorimetry

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Topical pain relievers are popular OTC products since they offer an instant analgetic action as well as comfortable feeling on the injured site, e.g., after a physical exercise. In order to achieve an immediate effect, the drug must be delivered rapidly into the deeper skin layers. Penetration enhancers are commonly incorporated to enhance drug permeation across the skin, typically via modulation of skin lipid organization [1]. Differential scanning calorimetry (DSC) can be applied to visualize the interaction of the formulation components with the skin lipids [2]. In addition, a fast and steady evaporation of the volatile components may improve patient compliance which is commonly sensed as “cooling”. The thermal gravimetric analysis (TGA) can be applied to investigate the evaporation profile of the formulations under study.

The formulations under study were doc® Ibuprofen Schmerzgel, Dolgit® Mikrogel, Ibutop® Gel, Ibutop® Creme, Voltaren® Emulgel and Voltaren® Schmerzgel. The evaporation profile of the formulation was examined at different temperatures between 25 and 40 °C. Some potential solvents of the topical formulations (water, dimethyl isosorbide, isopropyl alcohol and medium chain triglycerides) were also investigated. DSC measurement of pre-treated stratum corneum (with the formulation) was conducted within the temperature range of 20-120 °C with a heating rate of 5K/min.

From all the tested formulations, there were three groups of formulations with comparable evaporation rates each, i.e., Voltaren® products with 84% loss, the remaining gel products with 70% loss and Ibutop® Creme with the lowest loss of 25% (all after 60 min, 37 °C). The film/ residue of the formulation may still contain moisture and this is measurable by means of Karl-Fischer titration. The film of doc® Ibuprofen Schmerzgel, e.g., contained about 1.5% water. The evaporation rate of the solvents (at 25 and 37 °C) was isopropyl alcohol> water> dimethyl isosorbide> oil. This result shows that alcohol is responsible for a fast, initial cooling while sustained cooling is then kept by water. DSC measurements showed that the three gel formulations (doc® Ibuprofen Schmerzgel, Dolgit® Mikrogel and Ibutop® Gel) revealed a comparable interaction on the skin lipids. A weaker interaction was shown by Voltaren® Schmerzgel and Ibutop® Creme, respectively.

Acknowledgement
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Wissenschaftliche Posterausstellung: Poster 10

Interaction of Nanoparticles with Skin: Accumulation on Skin Surface and Drug Delivery or Translocation and Cellular Uptake

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Particulate carrier systems have been developed and are under investigation for skin and transdermal drug delivery. In order to obtain the desired drug release properties, investigations on particle-skin interactions should include the effects of particles on skin physiology and cell viability as well as the effect of skin on particle integrity, colloidal stability, drug delivery. Upon application of nanoparticles on skin surface, topically applied particles come in contact with stratum corneum, sweat glands and hair follicle canals which have a hydrophobic environment, and a number of lipids and proteins which might interact with the particles and influence their drug release properties. On the other hand, translocation of particles through the skin barrier might also take place along with uptake by different cell populations in the skin, e.g. keratinocytes, antigen-presenting cells. Using human skin explants models, we found a bright spectrum of particle-skin interactions depending on particle size, composition, rigidity, and coating. Rigid inorganic particles, like gold particles with silica shell with size of 160nm, accumulated in skin furrows and hair follicle canals without translocation to the viable epidermis. On the contrary, 200nm solid polystyrene nanoparticles translocated through the skin barrier and were found in association with skin immune system cells. Soft poly-lactic acid (PLA) particles, lost their supramolecular organization and released the incorporated fluorescence dyes in a time-dependent manner upon application on skin surface. PLA particles coated with the HIV-1 p24 peptide were found to release the adsorbed peptide only after their penetration in the hydrophobic environment of the hair follicle canal. The released peptide translocated into the epidermis and was found in association with keratinocytes and dendritic cells. On the contrary, biological virus-like particles carrying the same HIV-1 p24 peptide were found to be associated

prevalently with CD1a-positive Langerhans cells after transcutaneous application on skin explants. These findings show that nanoparticles are attractive drug delivery systems for dermatology and that a variety of drug release features can be achieved by tuning the physico-chemical properties of the nanoparticles in accordance to the skin environment.

Wissenschaftliche Posterausstellung: Poster 11

^{19}F-NMR for drug quantification in tape stripping experiments

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Introduction

Tape stripping is a well-known method to characterise the penetration of a drug into the stratum corneum. While the amount of corneocytes on the tape strips can easily be quantified by NIR or UV/Vis [1], the amount of active drug is usually determined by HPLC. In the present study, we employed 19F-NMR experiments for a quantitative analysis of flufenamic acid.

Experimental methods

Formulations

Microemulsions based on natural surfactants were developed. The formulations contained lecithin, sucrose laurate, alkylpolyglycoside or a mixture thereof as surfactant. Isopropanol served as co-surfactant. Moreover, the formulations contained water and neutral oil. Flufenamic acid served as model drug. 3% of the active substance was incorporated into the microemulsions.

Tape stripping procedure

Tape stripping experiments were executed on full-thickness porcine ear skin. Hair was carefully removed by clipping. Subsequently, 5 mg cm^{-2} of the formulation was applied. After a residence time of one hour, twenty tape strips were torn off at a defined spot and analysed for their content of drug and corneocytes. The quantification of the corneocytes was performed by NIR, while the drug content was analysed by NMR and HPLC after extraction with deuterated methanol.

NMR analysis

NMR experiments were performed on a BrukerAvance DRX 600 NMR spectrometer (BrukerBioSpin GmbH, Germany) operating at 564.69 MHz for ^{19}F as described in Schwarz et al., 2011 [2]. A 5 mm quadruple observe probe equipped with z-axis gradient coil was used. All measurements were done in deuterated methanol at a temperature of 298 K. Typical acquisition parameters chosen are: 15 ppm spectral width, 32000 data points, 90° excitation pulse, 2 s acquisition time, 1000 scans. The spectra were referenced externally (^{19}F: CCl_3F δ = 0 ppm). The processing and the analysis of the NMR spectra were done within the Topspin Software, Version 3.0 (BrukerBioSpinGmbH). For the processing the amount of data points was doubled by zero filling and an exponential window function with a line broadening factor of 3 was applied. To improve the accuracy of the integration the signals were deconvoluted by fitting a Lorentzian line shape.

In order to obtain the absolute concentrations the intensities from the integration were referenced to a calibratrion series, which was measured in the range from 0.5 to 1000 µg ml^{-1}.

Results

It was possible to determine the amount of flufenamic acid by ^{19}F-NMR (Figure 1). Moreover, the obtained results showed a highly linear correlation (R2=0.988) with the drug amounts determined by HPLC (Figure 2). Therfore, it could be shown that ^{19}F-NMR is highly suitable for the quantitative analysis of fluorinated drugs in tape stripping experiments.

Figure 1: Amount of flufenamic acid on tape strips quantified by ^{19}F-NMR

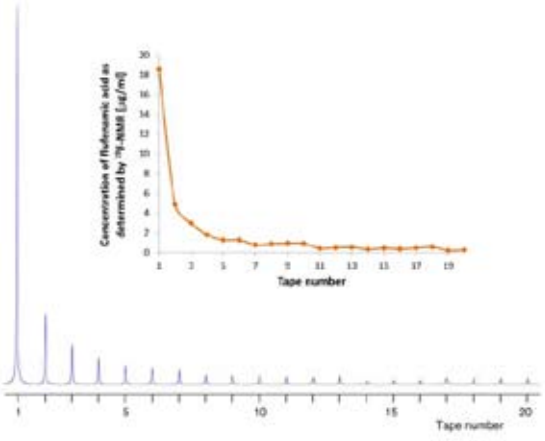
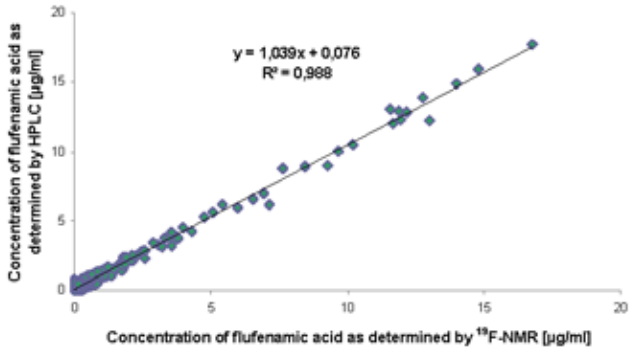


Figure 2: Linear correlation of the amounts of flufenamic acid on tape strips as determined by by ^{19}F-NMR and HPLC



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Wissenschaftliche Posterausstellung: Poster 11

Current state of development of human polymerase α inhibitors as innovative tumour therapeutics

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Actinic Keratosis (AK) is considered to be the most frequent carcinoma in situ today. AK lesions commonly occur on sun-exposed areas of the skin and organ transplant recipients with AK have the highest risk to develop invasive squamous cell carcinoma (SCC)[1]. Current AK therapy is limited either due to adverse drug reactions and/or limited efficacy. Hyperkeratosis, frequently associated with AK, reduces drug penetration to the target site.

Human polymerase-α inhibitors, identified by molecular modelling[2,3], outperformed the current standard for AK therapy, 5-fluoruracil, when tested in the tumour cell line SCC25, while not or at least less affecting normal human keratinocytes[4]. In order to achieve satisfactory penetration into skin of the most selective human polymerase-α inhibitor OxBu, the agent was loaded to solid lipid nanoparticles (SLN) with glyceryl behenate as lipid phase. The dispersion containing DMSO as penetration enhancer was embedded in a hydroxyethyl-cellulose gel matrix. In vitro release studies indicated prolonged OxBu release.

Based on the 3D SCC construct developed by Hoeller Obrigkeit and co-workers [5] an AK-like construct was grown in our laboratories and characterised. SCC12 cells forming nests were detectable in particular in the epidermis. OxBu induced a reduction in Ki-67 (proliferation marker), cytokeratin-10, AxL (SCC markers), MMP2 (invasion marker), while caspase-7 was activated (apoptosis marker). Aiming to substantiate the mode of cell death, the secretion of total cytokeratin-18 (marker for necrosis and apoptosis) and its caspase cleaved product (marker for apoptosis) were investigated by ELISA following OxBu exposure for up to 7 days. OxBu 0.05% solution appeared to be at least equipotent compared to solutions of both 5-fluorouracil 0.1% and aphidicolin 0.025%. The former being the gold standard of current AK therapy, the latter being frequently used as standard inhibitor of human polymerase alpha and delta for in vitro testing, however, failed to be introduced into clinical use. Taken together, OxBu may offer a novel approach for actinic keratosis therapy.

References

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Nanotoxicological Classification System (NCS) – a rational approach to assess the safety & risk of nanoparticles

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There is an increasing and by now almost uncontrolled use of nanomaterials in many different industrial areas, ranging from nanopigments in colours, via carbon nanotubes in cars to nanoparticles in cosmetics and pharma. In parallel there is an increasing concern by the consumers about the safety of these nanomaterials and potential risks for health and environment. Especially the popular press likes it to increase its circulation by lurid headlines about “dangerous nano”. As a consequence the public perception is getting more and more negative about nano, potentially impeding useful nanotechnology. The more it is necessary to put the safety and risk evaluation of nanomaterials on a scientific basis. This is done by the proposal of the nanotoxicological classification system (NCS) (1) for cosmetics and pharma, but it has also implications for other consumer products (e.g. personal care, nutrition).

The NCS places the nanomaterials in 4 groups of no or very little (I) via medium (groups II and III) to potential higher risk (IV). This is very important because it provides a guide on which nanomatreials toxicity studies should focus first. Meanwhile there are thousands of nanomaterials around, a safety evaluation for one material involves extensive different studies, but there is a limited capacity available to do these studies. Thus one should focus first on particles with potential highest risk, the NCS can be used as selection tool.

Toxicity determining parameters are size, degradability and biocompatibility. Depending on the size, the particles have different access to cells. Nanoparticles >100 nm to 1,000 nm have access to only a limited number of cells (macrophages), are therefore less risky, whereas nanoparticles < 100 nm can be internalized by any cell via endocytosis. Biodegradable particles will eventually disappear, most likely also a related undesired effect, non-biodegradable particles can stay forever in the body, thus being of higher risk. This results in 4 classes: I - >100 nm, biodegradable, II - > 100 nm, non-biodegradable, III - < 100 nm with easy cell access but still biodegradable, IV - < 100 nm and not biodegradable (= potentially highest risk, but not necessarily toxic!).

Another important point is the biocompatibility (B) or non-biocompatibility (NB). A non-biocompatible surface of a particle, even when the particle is of class I, might activate the immune system by opsonin adsorption. Therefore for a full picture each class needs to differentiate, resulting in a total of 8 classes from I-B, I-NB to IV-B and IV-NB. The classes can

also be shown in form of a traffic light system: green (I), yellow (II, III) and red (IV), easy to understand by politicians and the average consumer.

References

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Wissenschaftliche Posterausstellung: Poster 13

Anti-oxidative and skin protective effects of drug nanocrystals

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Drug nanocrystals are particles of an active reduced in size to the nanometer range. Due to their small size they possess many special nano properties compared to drug crystals in the micrometer range. Examples are increased dissolution velocity dc/dt, increased saturation solubility cs, and consequently an increased concentration gradient at membranes (e.g. skin), leading to enhanced penetration/permeation. Therefore, nanocrystals are a smart delivery system for poorly soluble pharmaceutical and cosmetic actives.

Dermal application of antioxidants is frequently used to scavenge free radicals in the skin and thus to protect the skin from damages caused by such free radicals. Antioxidants are therefore not only important in anti-aging, but also, in prevention of skin cancer. However, many antioxidants, especially secondary plant metabolites such as flavonoids, are poorly soluble and thus showing no or insufficient penetration into the skin. To assess the penetration enhancing effect, nanocrystals of the poorly water soluble flavonoids (Rutin and Hesperidin) were produced. In a human study, their antioxidant capacity was compared to a water soluble Rutin derivative (rutin with attached glucose). To assess the increase in “bioactivity” in the skin, not concentrations of the active were measured but the pharmacological effect. i.e. the achieved antioxidative activity (protection against UV light, increase in sun protection factor SPF). In addition, the skin protective effect was assessed in vivo by comparing biopsies of healthy, non-irradiated skin with those irradiated with UV light (non-treated, damaged) and irradiated skin treated before with drug nanocrystals.

The in vivo experiments showed up to a 1,000 fold higher activity of the nanocrystals compared to the water soluble rutin derivative (2 fold increase in SPF at 1/500 lower dissolved concentration of active). The skin damaging effect caused by UV was shown by a significant change in the morphology of the Langerhans cells in the biopsy of the irradiated, non-treated skin. The damaging effect was avoided when the skin was treated with nanocrystals prior to the UV irradiation.

In conclusion, nanocrystals can be used as a principal formulation strategy to improve the dermal activity of poorly soluble compounds. The activity increase found let to the development of cosmetic products with rutin nanocrystals (Juvena, line JUVEDICAL) and

hesperidin nanocrystals (la prairie, platinum rare). The same principle can be applied to poorly soluble drugs to increase their dermal activity.