P229 TOPICAL APPLICATION OF LIPOSOMALLY ENCAPSULATED CICLOSPORIN: AN IN VIVO / IN VITRO CORRELATION IN HAIRLESS MOUSE

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The kinetics of deposition of ciclosporin (CSA) into the dermis of hairless mouse skin after topical application of liposomally encapsulated ciclosporin, was determined using both ***in vitro*** and ***in vivo*** experiments. The overall mass balance was much lower for ***in vivo*** studies than for ***in vitro*** studies suggesting that excess formulation may have been lost despite attempts to control the process by anesthetizing the animals periodically to minimize their movements. The recoveries were generally about 70% ***in vivo*** compared to about 90% ***in vitro***. The amounts of CSA found in the tape strippings were roughly similar for ***in vivo*** and ***in vitro*** topical applications at all time points studied. The drug content in the deeper skin strata, however, were very different. CSA deposition in the deeper skin strata 4 hr after topical application was significantly greater with the ***in vivo*** studies. Further, CSA levels decreased rapidly as a function of time ***in vivo*** whereas these levels increased steadily with time ***in vitro***. The ***in vivo*** drug profile may be due to the clearance of drug into the systemic circulation by way of the dermal vasculature. For the ***in vitro*** diffusion experiments, the diffusional resistance of the hydrated dermis to a very hydrophobic drug such as CSA preempts transport into the receiver and results in a build-up of CSA levels in the dermis. The results of this study also suggest that the state of hydration of the stratum corneum plays an important role in determining the kinetics of drug transfer from liposomes into skin.

P230 THE EFFECT OF LIPOSOMAL LIPID COMPOSITION AND CONCENTRATION ON THE DEPOSITION OF GAMMA-INTERFERON AND TRANSFORMING GROWTH FACTOR-ALPHA INTO THE SKIN

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Topical delivery of peptide drugs has gained increasing attention and is considered to be one of the beneficial routes for the delivery of these drugs in view of its by-pass of gastrointestinal degradation and better patient compliance. In the case of skin disorders, systemic delivery of peptides may be ineffective because of rapid clearance from the circulation and an inability to deliver the molecule to the appropriate cells. Peptides usually are large molecular weight drugs of hydrophilic nature which are not readily topically absorbed. The objectives of the study were to study the topical absorption of gamma-interferon and transforming growth factor-alpha (TGF-α) ***in vitro*** by using a liposomal formulation. This study also describes the evaluation of the effect of lipid composition and the concentration of lipids of the liposomes on the deposition of the growth factors into the various strata of hairless mouse and pig skin ***in vitro***, in an attempt to find an optimum formulation for optimal drug deposition. The deposition of drug and lipid into the deeper strata of skin upon application of dehydration/rehydration liposomes was examined using radiolabeled drug and lipid markers. The studies have shown that by using a liposomal formulation, it is possible to deliver peptide drugs to the stratum corneum, as well as to the deeper skin strata where the basal cell layers reside. The extent of deposition of hydrophilic drugs from liposomal preparations following topical application is dependent on a variety of factors, particularly the liposomal lipid composition, the drug to lipid ratio and follicular density of the application site. The drug deposition into the deeper skin strata (epidermis and dermis) was optimum for formulations where the drug/lipid ratio was high. For all formulations tested, deposition into the deeper skin strata was much higher for the animal species having a more significant follicular route. By using liposomal formulations it is thus possible to deliver these large peptide molecules into the skin where they can exert their pharmacological effect.

P231 AMIKACIN-LOADED LIPOSOMES - PRODUCTION, CHARACTERIZATION AND TREATMENT EFFICIENCY IN MYCOBACTERIUM AVIUM INTRACELLULARE COMPLEX (MAC) INFECTED MICE

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Amikacin, an aminoglycoside, is active in vivo against MAC infections [1]. The encapsulation of amikacin in liposomes increases the treatment efficiency [2]. Many published results using liposomes as vehicle for this drug lack of precise characterization data and little or no attention was given to an adequate pharmaceutical quality [3]. However it is a prerequisite for registration by the regulatory authorities. In this study general aspects of the influence of physico-chemical data of liposome formulation on treatment efficiency were investigated.

Production of liposomes was performed using an ethanol injection method. A phospholipid / stabilizer / alcohol mixture was injected into an aqueous drug solution at high speed stirring. This led to liposomes of 180nm to 250nm average diameter, accessible for sterile filtration. We adjusted the surface charge and the phospholipid bilayer rigidity (microviscosity) by additives, e. g. tocopherole acetate or retinol palmitate. Particle size and polydispersity index were measured by photon correlation spectroscopy, the zetapotential by laser doppler anemometry. Microviscosity was determined by fluorescence polarization measurements using diphenylhexatriene as indicator. These additives increased the phospholipid bilayer rigidity from 90mPas to a maximum of 160mPas. The higher rigidity improves physical stability and can reduce the diffusion of drug into the outer phase. Furthermore the pharmaceutical quality of drug-loaded liposomes was compared with a commercial fat emulsion for parenteral nutrition as far as particle size is concerned. The maximum droplet diameter and total number of large particles per volume unit were analyzed by Coulter Counter technique. The content of large particles, which can potentially block blood capillaries, was similarly low in liposome dispersion and commercial, registered emulsions.

Infection of mice by M. avium strain TMC 724 ($1x10^5$ CFU/mouse) was treated with a regimen of liposome-encapsulated amikacin. The suspension was administered i. v. three times weekly for two weeks. Bacterial counts and histological staining were performed on spleen, liver and lungs obtained 7 days after cessation of treatment. Two different stages of infection - one and five weeks after inoculation - were studied. Microbiological and histopathological differences could be detected depending on the drug formulation (liposome-entrapped vs. aqueous), its dosage and the grade of disease when treatment was started. Liposomal amikacin was superior in every aspect studied. Kinetic studies were initiated to quantify the prolonged release effect of liposomes and to optimize administration intervals.

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P232 GLYCOLIPIDS AS CRYOPROTECTANTS IN THE LYOPHILIZATION PROCESS OF LIPOSOMES

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The lyophilization technique seems to be the most promising way for the physical stabilization of liposomes. To prevent dehydration damages of functionality and integrity of membranes, they have to be protected by agents. Carbohydrates are the most effective cryoprotectants basing on their ability to interact via hydrogen bonds to the phospholipid headgroups.
We investigated the possible protective effects of carbohydrates directly linked to membrane surfaces by incorporating glycolipids of the general structure

$$H_{33}C_{16}\text{-}(O\text{-}CH_2\text{-}CH_2)_n\text{-}O\text{-carbohydrate}$$

n = 0,1,2,3 for glucosides and galactosides, n = 0 for cellobioside

into phospholipid liposomes before lyophilization. The ethoxy spacers should guarantee a modified location of the glycosidic headgroups in the phospholipid membrane.
On one hand, a local high concentration of carbohydrates on the polar region of phospholipids is achieved, and on the other hand , due to the fixed location of the glycosidic headgroups more insights into protective effects of carbohydrates should be possible.
The glycolipid containing vesicles were analytically analysed before and after lyophilization and following rehydration to characterize the role of glycosidic structures as protectors.
The results of Differential Scanning Calorimetry and Infrared spectroscopy of the dehydrated vesicles indicate the miscibility and interactions of the lipidic compounds in dehydrated state. No phase separations were detectable after rehydration too.
According to the results of a fusion assay employed resonance energy transfer the insertion of the glycolipids lowers the fusion rate of fluid phospholipid vesicles. The glycolipids depress vesicle fusion related to their headgroup size, but independent from the spacer induced headgroup location in the membrane, proved by interactions of these vesicles with lectins.
Considering the retention rate of entrapped hydrophilic solutions in the vesicles after lyophilization and rehydration it is evident, that the glycolipids can't protect the membrane's barrier function completely. But in the presence of free carbohydrates a hyperadditiv increase in stability of glycolipid containing vesicles occurs. On the basis of the increased aggregational behavior of glycolipid vesicles compared to pure phospholipid liposomes the hyperadditiv stabilization will be explained by changed bonding conditions to sugars in presence of glycolipids.
By that, the glycolipids get their importance as indirect cryoprotectants for lyophilized liposomes influencing the carbohydrate structure on vesicle surface.
The mechanisms of action and prospects of further application of glycolipids as membrane bound protectors will be discussed in detail.