P230 THE 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 stripings 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 h after topical application was significantly greater with the in vivo studies. Further, CSA levels decreased rapidly as a function of time in vivo whereas those 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.

P231 AMIKACIN-LOADED LIPOSOMES – PRODUCTION, CHARACTERIZATION AND TREATMENT EFFICACY IN MYCOBACTERIUM AVIUM INTRACELLULARE COMPLEX (MAC) INFECTED MICE
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Aminoglycoside, an aminoglycoside, is active in vivo against MAC infections [1]. The encapsulation of amikacin in liposomes increases the treatment efficacy [2]. Many published results using liposomes as drug 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 efficacy 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 vascular filtration. We adjusted the surface charge and the phospholipid bilayer rigidity from 90mPas to a maximum of 160mPas. The zetapotential by laser doppler anemometry. Microviscosity was determined by fluorescence polarization measurements using diphenylbexatriene as indicator. The additives, e.g. tocopherol acetate or retinol palmitate. Particle size and polydispersity index were measured by photon correlation spectroscopy, the polarization measurements using diphenylbexatriene 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 similarity low in liposome dispersion and commercial, registered emulsions.

Infection of mice by M. avium strain TM. 724 (1x10^7 CFU/mouse) was treated with a regimen of liposomes-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 (liposomes-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 extent of liposomes and to optimize administration intervals.


P232 GLYCOCARDS 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

\[ H_3C \overset{(O-CH=CH_2)}{\longrightarrow} O-carbohydrate \]

where \( n = 0,1,3 \) for glucosides and galactosides, \( n = 0 \) for cellobiose into liposomes before lyophilization. The ethyl spacer 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 glycolipidic structures as protectors.

The results of Differential Scanning Calorimetry and Infrared spectroscopy of the dehydrated vesicles indicate the miscibility and interactions of the lipid compounds in dehydrated state. No phase separations were detectable after rehydration too. According to the results of a frozen 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 solutes 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 hyperosmotic 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 hyperosmotic stabilization will be explained by changed binding conditions to sugars in presence of glycolipids.

By that, the glycoproteins get their importance as indirect cryoprotectants for lyophilized liposomes influencing the carbohydrate structure on vesicle surface.