Nano-formulation for topical therapy of skin precancerous lesions: in vitro, ex vivo and in vivo evaluation

Maria Natalia Calienni

Laboratory of Biomembranes - GBEyB (IMBICE, CCT-La Plata, CONICET), Department of Science and Technology, National University of Quilmes, Bernal (B1876BXD), Argentina

Ultradeformable liposomes (UL) are a drug delivery nanosystem with an elastic modulus lower than conventional liposomes. This feature makes UL capable to penetrate the stratum corneum (SC) of the skin and release their content into the viable epidermis, where neoplastic events occur in skin cancer. 5-Fluorouracil (5FU) is a classic antineoplastic drug, administered parenterally, with severe side effects. Therefore, the incorporation of 5FU in UL could improve specific-site delivery and aims to reduce side effects. In this work, a UL 5FU-loaded formulation (UL5FU) of soy phosphatidylcholine and sodium cholate, as border activator, was obtained as a future topical treatment for skin precancerous lesions.

The nano-formulation was biophysically characterized in size, encapsulation efficiency, stability in time, lamellarity, drug release, deformability, drug to lipid ratio and drug-membrane interaction.

Penetration properties were studied on a Saarbrücken Penetration Model device with human skin explants. The skin penetration was determined by tape stripping technique, removing each layer of SC and analyzing the presence of the drug. Also, skin was incubated with UL5FU with two fluorescent labels, Rhodamine-DPPE for the tracking of membrane lipids and FITC for the tracking of aqueous content. Intact skin and transversal sections of 20 µm in thickness were studied by confocal laser scanning microscopy.

In vitro studies were carried out in two human cell lines: HaCaT (non tumoral) and SK-Mel-28 (melanoma derived). Cytotoxicity was studied by MTT, Crystal Violet and Neutral Red at 4 and 24 h. Uptake of UL5FU with Rhodamine-DPPE and FITC was analyzed at 4 and 37 ºC in both lines. Induction of apoptosis after 6 h of incubation was assessed by flow cytometry using a kit of Annexin V conjugated with FITC.

In vivo studies in zebrafish (Danio rerio) larvae (4-7 days post-fecundation) were performed to determine toxicologic and teratogenic effects of 5FU and UL5FU. Zebrafish is an increasingly accepted animal model for nanotoxicological studies because it high correlation effects and other advantages if compared to other animals. Effects were assessed by alterations in the swimming activity, alterations in the heart rate, morphological changes and histological analysis of brain -particularly the raphe populations-, spinal cord and liver in parasagittal serial sections of 10 µm thickness staining with hematoxylin-eosin.

The UL5FU formulation was stable over time and incorporation of 5FU did not alter significantly its deformability properties, although it interacts with the liposomal membrane. It was capable to penetrate the SC and deliver 5FU in the viable epidermis of intact skin. UL5FU strongly increased the cytotoxicity in the tumoral line after 24 h of treatment. Furthermore, SK-Mel-28 showed a higher uptake than HaCaT and apoptosis studies showed a differential effect between both lines. After 6 h of incubation there was significant induction of apoptosis only in the tumor line by UL5FU (24.53%) and 5FU (16.49%). From in vivo studies, valuable toxicological and teratogenic information was obtained. UL5FU and 5FU produced alterations in the swimming activity -but at a very different range of doses-, which could be related to neurological damage, and induced morphological changes in larvae. Also, cardiological effects were observed and could be related to a secondary effect of the drug, because the heart is a target organ for it.

The research continues to develop a novel nano-formulation of UL as delivery system of the new drug Vismodegib for topical treatment of skin precancerous lesions.

References