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Vismodegib-loaded nanoformulation for topical skin cancer therapy: reducing drug amounts while reaching supra-therapeutic concentrations

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Basal cell carcinoma (BCC) is the most common type of skin cancer, representing 80% of all cases. Genetic alterations leading to aberrant constitutive activation of the Hedgehog (Hh) signaling pathway are associated with the development of most BCC. Vismodegib (Erivedge®, Genentech) is a first-in-class selective inhibitor of the Hh signaling pathway approved by the US Food and Drug Administration in 2012 for the treatment of locally advanced and metastatic BCC. Currently, the treatment with Vismodegib consists of the daily oral administration of Erivedge® capsules. However, there are several side effects associated with the systemic administration of this active principle that frequently causes patients to discontinue treatment. On the other hand, topical drug-delivery shows potential over systemic delivery in the treatment of dermatological diseases due to the possible reduction of side effects and the increment of the local drug concentrations. However, the *stratum corneum* (SC) of the human skin forms an effective barrier. Particularly, nanomedicine provides tools to overcome the SC barrier which allow the targeted transport of actives through nano-drug delivery systems. One of the lipid-based nanosystems that has successfully achieved the transport of actives through the SC to deeper layers of the skin are the ultradeformable liposomes (UDL). UDL are highly elastic liposomes, which can penetrate across the SC at the body temperature, impelled by the transdermal hydration gradient and dehydration pressure. Therefore, this work aimed to obtain and characterize Vismodegib-loaded UDL (UDL-Vis) as a potential topical therapy against BCC.

UDL-Vis were prepared with soy phosphatidylcholine and sodium cholate, and the obtained formulation was characterized by several techniques, both experimental and *in silico*. We have determined the mean size, ζ potential, stability over time, liposomal deformability after incorporation of the drug, and we have characterized the interaction between Vismodegib and the liposomal membrane. Moreover, *in vitro* penetration of UDL-Vis in human skin was assessed with the Saarbrücken Penetration Model. UDL-Vis cytotoxicity, cellular uptake, and the induction of apoptosis were tested in two human cell lines (HaCaT and SK-Mel-28), which present the Hh pathway activated. Finally, the toxicity of UDL-Vis was tested *in vivo* in zebrafish (*Danio rerio*) larvae. We have chosen zebrafish as an intermediate model between *in vitro* determinations and *in vivo* studies with mammals because it is a growing model in the field of nanotoxicology.

We have obtained a nanoformulation with high encapsulation efficiency, achieving a concentration in the viable epidermis of human skin almost thrice higher than the required dose for BCC treatment, employing around 2500 less drug than an oral dose. Remarkably, the incorporation of Vismodegib to UDL increased its cytotoxic effects, induced a higher rate of apoptosis, and caused effects at lower concentrations than the free drug *in vitro*. Besides, this work brings new information about the toxicological effects of Vismodegib and this nanoformulation in zebrafish, data which are important for further studies in a murine model of BCC. In sum, UDL-Vis could not only allow the topical delivery of the drug non-invasively but also enhance the performance of the drug due to a possible synergy between the liposomal matrix and Vismodegib.

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