OC5: Identification of keratinocytes' interaction with nanostructured lipid carriers

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Introduction: The keratinocytes are important cells in the context of psoriasis. Lipid nanoparticles are being used to improve the topical treatment, as drug carriers. Nanostructured lipid nanocarriers (NLC) are lipid nanoparticles with enhanced skin compatibility. The study of nanoparticles internalization and interaction with the keratinocytes is important to predict and modulate drug efficiency and therapeutic outcome.

Objectives: The goal of this study is the identification of the keratinocytes' interaction with lipid nanoparticles loaded with methotrexate and their biological activity.

Materials and Methods: NLC were applied to deliver methotrexate to the keratinocytes (HaCaT human cell line), were synthesized through hot ultrasonication and physico-chemically characterized. The cellular uptake and endocytosis' studies were made by flow cytometry using NLC loaded with coumarin 6. In the endocytosis' study, inhibitors were used to identify the uptake mechanism: active transport, clathrin and caveolae-mediate endocytosis and macropinocytosis. The study of exocytosis, transcytosis and paracytosis were conducted using a transwell system after HaCaT monolayer differentiation through high calcium concentration.

Results and Discussion: NLC had a mean diameter of 245 nm, spherical shape, negative zeta potential and polydispersity index lower than 0.15. Their cellular uptake was time dependent, increasing slightly with the time. The incubation at 4ºC and with sodium azide showed an energy dependency in the endocytosis' process, which was mediated by clathrin and macropinocytosis. The endocytosis' inhibition was more pronounced with the nanoparticles made of cetyl-palmitate as solid lipid. Upon internalization, 10% of the NLC were discharged by exocytosis and/or trancytosis mechanisms.

Conclusion: In summary, it was concluded that the internalization of the nanoparticles required energy and involved clathrin-mediated endocytosis and macropinocytosis. The results obtained allowed a better understanding of the uptake and interaction of NLC with human keratinocytes and demonstrated the good viability of the carrier for skin drug delivery (major percentage of the drug remained within the cell).

References

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