Flow cytometry and live confocal analysis used to evaluate the uptake and the intracellular distribution of FITC-ODN into keratinocyte cell line

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Purpose: To investigate the mechanism of the internalisation and the cellular distribution of 5' fluorescein conjugated PS-ODN (FITC-ODN) after transfection with different lipidic vesicles/oligo complexes.
Methods: MLV liposomes were prepared with one of the most used cationic lipid (DOTAP) and different amount of a cholic acid (UDCA) to release the oligo into HaCaT cells. Using flow cytometry, the cellular uptake of the oligo was studied with and without different inhibitors able to block selectively the different pathways involved in the internalisation mechanism. The intracellular distribution of the oligo was analysed by confocal microscopy treating the cells with the liposome/oligo complexes and directly observing without any fixing procedure. To better carry out the co-localization studies, fluorescent labelled markers, specific for the different cellular compartments, were co-incubated with FITC-ODN.
Results: At the beginning, the different lipidic vesicles affect the internalisation mechanism of FITC-ODN. After using the inhibitors, the uptake of complexes involved a different internalization mechanism. The live confocal microscopical analysis demonstrated that, after 1h from the complex incubation, the oligo was transferred into cells and localized into the endosomes; after 24 h, oligo was intracellularly localized close to the nuclear structure in a punctuate pattern. However, the results from fusion experiments showed also a binding of a quite amount of oligo with the cell membranes.
Conclusion: When complexed with lipidic vesicles, the oligo was internalised into HaCaT cells by different endocytotic mechanisms as demonstrated by flow-cytometry and co-localization studies. The different inhibition mechanisms were correlated to the different composition of lipidic vesicles used as carriers.