Cationic liposomes carrying siRNA: impact of lipid composition on physicochemical properties, cytotoxicity and endosomal escape

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In recent year, cationic liposomes have gained a lot of attention for siRNA delivery using different local routes of administration as the vaginal [1] or the pulmonary routes. However, lipoplexes have to face several biological and intracellular barriers before releasing the genetic cargo. In this study, we focus our effort on intracellular barriers and more specifically on endosomal escape and cytosolic delivery of siRNA as well as on the cytotoxicity due to cationic lipids. Indeed, we have previously demonstrated that the surface charge of liposomes composed of the cationic lipid DOTAP is correlated to the induction of cytotoxicity [2]. In the present study, we have investigated the impact of different cationic lipids and co-lipids on the cytotoxicity and also on the endosomal escape of siRNA by flow cytometry, qRT-PCR and Western Blot assays [3]. To address these issues, we developed four liposomal formulations composed of two different cationic lipids (DOTAP and DC-Cholesterol) and different ratio of co-lipids (cholesterol and DOPE). Formulations were DOTAP/Cholesterol/DOPE 1/0.75/0.5, DOTAP/Cholesterol/DOPE 1/0.5/0.5, DOTAP/DOPE 1/1 and DC-Cholesterol/DOPE 1/1.

Each type of liposomes were complexed to siRNA at six different N/P molar ratios and physico-chemical properties were characterized in terms of Z-average size, Zeta potential and complexation efficiency by gel retardation assay. Consequently, three N/P ratios (2.5, 5 and 10) were selected for *in vitro* experiments on A549 cells.

First, we studied the cell viability of A549 cells treated during 24 h with liposomes complexed to inactive siRNA at different N/P molar ratios at siRNA concentrations of 40 and 100 nM. We have shown that the cytotoxicity is influenced by the N/P ratio, the concentration of cationic lipid as well as the nature of the cationic lipid. Secondly, the cellular uptake were evaluated by flow cytometry using the dry Trypan Blue® to quench the external fluorescence. Despite the fact the transfection rate were not significantly different, the mRNA knock-down efficiency were not similar between formulations. Liposomes containing 50% of DOPE induced a mRNA silencing of around 80% as well as the protein knock-down. This study allowed to highlight crucial parameters in order to develop lipoplexes which are safe and induce an efficient intracytoplasmic release of siRNA.

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