Development and optimization of pegylated lipoplexes for vaginal application

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**Purpose**

In the field of nanoparticles, cationic liposomes are promising strategy to protect and transport siRNA into the cytoplasm. The resultant cationic nanovectors containing both siRNA and liposomes are called lipoplexes. In this study, lipoplexes are developed in the context of cervical cancer. Human Papillomaviruses (HPV16 essentially) are responsible for this cancer by the overexpression of two oncoproteins, E6 and E7. They are essential players in order to immortalize keratinocytes by decreasing tumor suppressor genes (p53 and pRb).

The aim is to develop pegylated lipoplexes anti-E6 for an intra-vaginal treatment of cervical cancer. The addition of polyethylene glycol (PEG), a hydrophilic polymer), is essential to decrease adhesive interaction with cervico-vaginal mucus. Influences of molecular weight and density of PEG on physico-chemical properties and *in vitro* response will be considered.

**Methods**

DOTAP/Cholesterol/DOPE liposomes (1/0.75/0.5) prepared by the hydration of lipidic film method were mixed with siRNA (100nM) at N/P ratio of 2.5. PEG 750 or PEG 2000 was grafted on lipoplexes by the post-insertion process. Density of both PEG was 10%, 30% and 50% / mole of DOTAP. Z-Average diameter, PdI and zeta potential were analysed by Malvern Nano ZS and percentage of encapsulation was determined by Quant-iT™ Ribogreen® RNA Assay. Transfection capacity of lipoplexes was analysed by flow cytometry, 24 hours post-transfection on CaSki cells (HPV16+). Finally, activity was determined by qRT-PCR on mRNA E6 extinction and by western blot on p53 re-expression, 48 hours post-transfection.

**Results**

The size of unpegylated lipoplexes is 216 nm with a PdI < 0.2. The size increases when PEG 750 was added and decreases with PEG 2000. Moreover, above 10% of both PEG nanoparticles are too much polydispersed. Zeta potential of unpegylated lipoplexes is +44mV and logically decreases with PEG density. 93% of siRNA are entrapped in cationic liposomes. This percentage of encapsulation slightly decreases with the addition of PEG 750 contrarily to the addition of PEG 2000, which induces a greater decrease in the percentage of encapsulation. All formulations with or without PEG induce more than 75% of transfection. Pegylated lipoplexes induce a higher level of transfection with a higher MFI (Mean Fluorescence Intensity). Concerning mRNA E6 extinction, Oligofectamine® is more efficient than unpegylated lipoplexes (respectively 60% and 78% of remaining mRNA E6). There are no statistical differences between lipoplexes with and without PEG. Despite qPCR differences, western blot reveals the same re-expression of p53 for both lipoplexes and Oligofectamine®.

**Conclusion**

These lipidic nanoparticles show appropriate physicochemical characteristics even if with high density of PEG, heterogeneity increases and encapsulation of siRNA decreases. All formulations allow transfecting more than 75% of cells with high MFI and induce better transfection results than Oligofectamine®. However these results do not result in a higher extinction of mRNA E6. Moreover, the difference observed in qRT-PCR between lipoplexe E6 and Oligofectamine® E6 is not confirmed in terms of p53 re-expression. This result needs to be verified for pegylated formulations. If no differences of p53 re-expression are observed, pegylated formulations will be selected to favour mucus diffusion.