Promoting vaginal distribution of two active siRNA-complexed in liposomes for cervical cancer treatment

Anna Lechanteur[1,2],, Tania Furst[1], Brigitte Evrard[1], Philippe Delvenne[2], Pascale Hubert[2], Géraldine Piel[1]

[1] Laboratory of Pharmaceutical Technology and Biopharmacy, CIRM, University of Liege, Liège, Belgium

[2] Laboratory of Experimental Pathology, GIGA-CANCER, University of Liege, Liège, Belgium

Contact : anna.lechanteur@ulg.ac.be

The use of small interfering RNA (siRNA) is a great promise for the treatment of different mucosal cancers but their physico-chemical properties make these administration challenging. The complexation of siRNA with liposomes and polyethylene glycol (PEG) is actually the best strategy to develop muco-penetrating nanoparticles. However, the addition of PEG may interfere with the transfection process of lipoplexes and with the releasing of siRNA into the cytoplasm due to a steric hindrance. The aim of this project is to determine which type and which density of PEG allow to develop an efficient and an atoxic lipoplexes which can also penetrate through the vaginal mucus. In this this study, two active siRNA are selected in the context of cervical (pre)neoplastic lesions induced by the infection of different high-risk Human Papillomavirus (HR-HPV).

PEGylated lipoplexes were made of DOTAP/Cholesterol/DOPE (molar ratio 1/0.75/0.5) liposomes and complexed with siRNA at a N/P ratio of 2.5. DSPE-PEG_{2000} and Ceramide-PEG_{2000} were added by post insertion technique (20-30-50% of DOTAP in molar ratio). Physico-chemical properties were characterized in water as well as in mucin solution, and mRNA knockdown were studied by qRT-PCR. Biological activities of siRNA-antiE7 and anti-MCL1 were tested on different HPV16 and HPV18 cell lines. Then, the penetration and efficiency of formulations on a 3D-cervical model lesion were performed. Finally, PEGylated lipoplexes were administrated into the cervical vaginal tract of female C57Bl/6 mice to study the muco-penetration *in vivo*.

In this study, we have found that both the type and the percentage of PEG added around lipoplexes are crucial factors in order to develop efficient lipoplexes. The Ceramide-PEG_{2000} (20%) was selected and induced a significant mRNA knockdown. This formulation induces the decreasing of proliferation and the induction of apoptosis of HPV16 and HPV18 positive cells. After validating the colloidal stability of PEGylated lipoplexes in mucus, the penetration and the delivery of siRNA into different mucosal models have also been validated. Moreover, this formulation was effective on a 3D-cervical lesion model HPV16 positive. Since the vaginal administration of this nanoparticle allows a complete coverage of the mucosal epithelium, we propose this nanocarrier as a promising therapeutic candidate for a wide range of HPV-induced mucosal cancers.

References

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