Cationic Lipid-Detergent Conjugates as New Reagents for siRNA Delivery

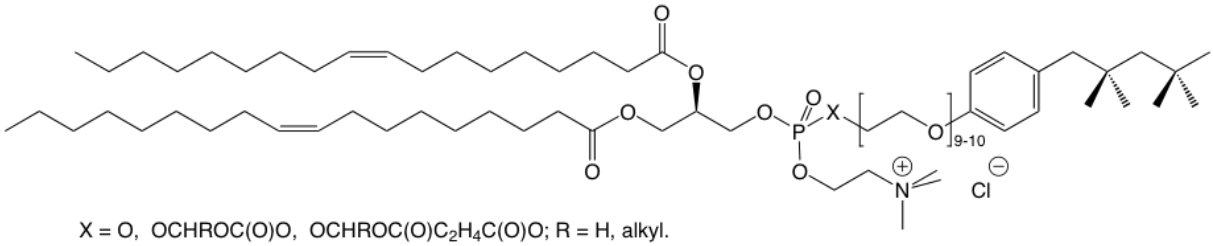
Philippe Pierrat, Françoise Pons, Guy Zuber, Luc Lebeau

Laboratoire de Conception et Application de Molécules Bioactives,
CNRS - Université de Strasbourg, Faculté de Pharmacie, Illkirch, France
llebeau@unistra.fr

One of the potential benefits of drug delivery systems in medicine is the creation of nanoparticle-based vectors that deliver therapeutic cargo in sufficient quantity to a target site to enable a selective effect, width of the therapeutic window depending on toxicity of the vector and cargo. In this work, we intended to develop a new kind of nucleic acid carriers which are the result of the conjugation of DOPC with Triton X-100®.

Cationic lipids were introduced more than twenty years ago to deliver DNA molecules to cells. Since then, they became useful tools for biomedical research and have been adopted as well for siRNA delivery. Their mode of action is based on the property of adherent cell lines to easily internalize large quantities of cationic complexes in endocytic compartments and on the incorporation of membrane-perturbing elements within internalized complexes for triggering subsequent release of the nucleic acid payload into the cytosol. The high membrane disruptive properties of detergents have been thus early considered for improving nucleic acid delivery. However, the use of such compounds remains delicate because of the rapid exchange of the detergent molecules between the complex and the aqueous environment (resulting in an irreversible decondensation of the nucleic acid even before entry into the cells) and direct plasma membrane permeation that may have an irreversible impact on cell viability.

We hypothesized that conjugation of a cationic lipid to a membrane active species might offer interesting properties as the detergent cannot be depleted from the transfection particles. Thus the membrane phospholipid DOPC was conjugated to Triton X-100® through spacers displaying various resistance to chemical hydrolysis and enzyme degradation to manage with biodegradability. The resulting cationic conjugates were evaluated in *in vitro* siRNA delivery experiments and showed that they can deliver siRNA to cells with remarkable efficiency. The initial phosphoester bond can be replaced with the phospho(alkyl)ene-carbonate group with no loss in transfection activity while the associated cytotoxicity was significantly decreased, as assessed by metabolic activity and membrane integrity measurements. Toxicity of the conjugates incorporating a phospho(alkyl)enesuccinnate moiety proved even lower but was clearly balanced with a reduction of the siRNA delivery efficiency. Otherwise, the phospho(alkyl)enecarbonate conjugates revealed some hemolytic activity whereas the parent phosphoester did not. The raison why these conjugates behave differently with respect to hemolysis might be a consequence of unusual fusogenic properties and probably reflects the difference in the stability of the conjugates in the intracellular environment.



X = O, OCHROC(O)O, OCHROC(O)C_2H_4C(O)O; R = H, alkyl.