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Switchable Lipids: From a Molecular Switch to a pH-Sensitive System for the Drug and Gene Delivery

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Abstract : Although several products have reached the market, gene therapeutics are still in their first stages and require optimization. It is possible to improve their lacking efficiency by the use of carefully engineered vectors, able to carry the genetic material through each of the biological barriers they need to cross. In particular, getting inside the cell is a major challenge, because these hydrophilic nucleic acids have to cross the lipid-rich plasmatic and/or endosomal membrane, before being degraded into lysosomes. It takes less than one hour for newly endocytosed liposomes to reach highly acidic lysosomes, meaning that the degradation of the carried gene occurs rapidly, thus limiting the transfection efficiency. We propose to use a new pH-sensitive lipid able to change its conformation upon protonation at endosomal pH values, leading to the disruption of the lipidic bilayer and thus to the fast release of the nucleic acids into the cytosol. It is expected that this new pH-sensitive mechanism promote endosomal escape of the gene, thereby its transfection efficiency. The main challenge of this work was to design a preparation presenting fast-responding lipidic bilayer destabilization properties at endosomal pH 5 while remaining stable at blood pH value and during storage. A series of pH-sensitive lipids able to perform a conformational switch upon acidification were designed and synthesized. Liposomes containing these switchable lipids, as well as co-lipids were prepared and characterized. The liposomes were stable at 4°C and pH 7.4 for several months. Incubation with siRNA led to the full entrapment of nucleic acids as soon as the positive/negative charge ratio was superior to 2. The best liposomal formulation demonstrated a silencing efficiency up to 10% on HeLa cells, very similar to a commercial agent, with a lowest toxicity than the commercial agent. Using flow cytometry and microscopy assays, we demonstrated that drop of pH was required for the transfection efficiency, since bafilomycin blocked the transfection efficiency. Additional evidence was brought by the synthesis of a negative control lipid, which was unable to switch its conformation, and consequently exhibited no transfection ability. Mechanistic studies revealed that the uptake was mediated through endocytosis, by clathrin and caveolae pathways, as reported for previous lipid nanoparticle systems. This potent system was used for the treatment of hypercholesterolemia. The switchable lipids were able to knockdown PCSK9 expression on human hepatocytes (Huh-7). Its efficiency is currently evaluated on in vivo mice model of PCSK9 KO mice. In summary, we designed and optimized a new cationic pH-sensitive lipid for gene delivery. Its transfection efficiency is similar to the best available commercial agent, without the usually associated toxicity. The promising results lead to its use for the treatment of hypercholesterolemia on a mice model. Anticancer applications and pulmonary chronic disease are also currently investigated.

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