682. New Histidylated Cationic Lipids for DNA- and mRNA- Based Lipofection

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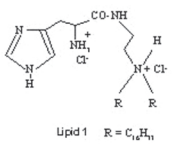
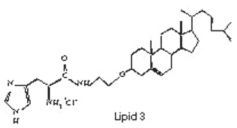
Plasmid delivery into the cytosol remains one of the limiting factor to achieve efficient transfection. We have previously demonstrated that the presence of endosome-disrupting multiple histidine functionalities in the molecular architecture of cationic polymers significantly enhances their gene delivery efficiencies. We designed and synthesized two novel non-glycerol and a cholesterol based histidylated cationic amphiphiles containing a single histidine head group. Physico-chemical characteristics of all the novel liposomes and lipoplexes including lipid:DNA interactions, global surface charge, sizes, etc. were evaluated. We found that L-histidine-(N,N-di-n-hexadecylamine)ethylamide (lipid 1) and L-Histidine(N,N-di-n-hexadecylamine, N-methyl)ethylamide (lipid 2) in combination with cholesterol and Cholesteryl-L-Histidine-Ethylamide (lipid 3) in combinaison with DOPE gave efficient DNA and mRNA transfections into various cell lines. DNA transfection efficiency into A549, 293T7 and HeLa cells of Chol/lipid 1 lipoplexes was similar with that of FuGENE6 and DC-Chol lipoplexes but was two order of magnitude higher in HepG2 cells. Membrane fusion activity measurements using FRET technique showed that the histidine head-groups of Chol/lipid 1 liposomes mediated membrane fusion in the pH range 5-7. By using the cytosolic luciferase expression vector (pT7Luc) under the control of the bacterial T7 promoter, we showed that the release of DNA from the endosomally trapped lipoplexes to the cytosol is acidic dependent and presumably mediated by the imidazole ring protonation of histidine head group

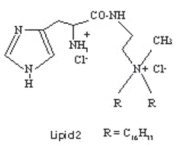
of these cationic amphiphiles. A better efficiency was obtained with Chol/lipid 2 lipoplexes than with Chol/lipid 1 lipoplexes when using the cytosolic luciferase expression vector. As anticipated, transfection efficiency of Lipid 3 was greatly inhibited in the presence of Bafilomycin A1. By contrast, endosome escape of DNA with a new cholesterol based cationic lipid containing no histidine head-group (Alanine-Cholesteryl-Ethylamide; lipid 4) seemed to be independent of endosome acidification. However, transfection efficacy of lipids 3 & 4 was similar. In conclusion, we show that covalent grafting of a single histidine amino acid residue to suitable twin-chain hydrophobic compounds or cholesterol is sufficient to impart remarkable transfection properties on the resulting cationic amphiphile via endosome-disrupting characteristics of the histidine functionalities.



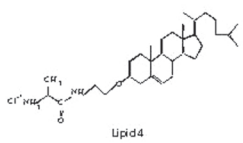
Lipid 1 $R = C_{16}H_{33}$



Lipid 3



Lipid 2 $R = C_{16}H_{33}$



Lipid 4