

## **GTS Journal Club: Conjugation of a Single Histidine Residue to Cationic Lipids for Enhancement of Gene Delivery**

Endosomal escape of cationic lipid/DNA complexes (lipoplexes) remains one of the more significant barriers to efficient lipid-mediated gene delivery. Endosomes, in fact, present a dual barrier to DNA translocation: 1) endosomes become highly acidic following fusion with lysosomes, leading to degradation of the transfected DNA, and 2) the endosomal membrane poses a physical barrier to moving DNA into the cytosol and subsequently towards the nucleus. Several approaches have been used to overcome endosomal obstacles to DNA delivery, the most popular being the use of neutral co-lipids, such as dioleoyl phosphoethanolamine (DOPE) and dioleoyl phosphatidylcholine (DOPC), in combination with the primary cationic lipid. These neutral co-lipids have been very effective for mediating efficient DNA transfection. In a recent issue of *Gene Therapy*, Kumar, *et al.* used an alternative approach to obtain a significant enhancement of gene delivery by addition of a single histidine residue to cationic lipids (1). The authors showed that the release of DNA from endosomal sequestration likely resulted from the addition of this histidine residue.

### **Preparation of Histidylated Cationic Lipids**

Two novel mono-histidylated cationic lipids, designated lipid 1 and lipid 2, were designed and synthesized. Both lipids contained a positive charged amine head group linked to two C<sub>15</sub>H<sub>31</sub> hydrocarbon chain tails. Single histidine residues were covalently conjugated to the amine head groups, and lipid 2 was further modified by the addition of a quaternary methyl group to the tertiary amine.

### **Determining Transfection Efficiency**

The transfection efficiency of the two lipids was initially determined by transfecting the human embryonic kidney cell line 293-T7, which constitutively expresses T7 RNA polymerase. Two different vectors were used for this experiment: a pCMV-luciferase vector for nuclear transcription and a pT7-luciferase vector for cytoplasmic transcription. Increasing molar ratios of cholesterol to lipid and charge ratios of lipid to DNA were tested for their effects on luciferase expression. The investigators found that expression was not affected by changing the cholesterol to lipid 1 molar ratios over the range of 1:2 - 3:1. Neither was transfection efficiency affected by varying the lipid 1 to DNA charge ratios over the range of 3.5 - 1.3. However, approximately 100 times greater luciferase expression levels were observed when transfecting the pCMV-luciferase plasmid compared to the pT7-luciferase vector. The authors propose that the lower expression level of the pT7 vector might be due to low levels of T7 polymerase expression or weak T7 promoter activity.

The investigators also found that the transfection efficiency of the pCMV-luciferase was enhanced significantly when the lipid:DNA charge ratio was increased. The luciferase expression was 10-fold higher at a lipid:DNA (N/P) charge ratio of 1.75 than at a ratio of 1. In contrast, the optimal expression level of pT7-luciferase was found at a charge ratio of 1. The authors suggest that the greater expression levels seen at higher lipid: DNA ratios for the pCMV plasmid might be due to a more significant degree of lipid-mediated protection of plasmid bound for the nucleus. However, the same high lipid:DNA ratios might result in limited access of the T7 polymerase to the pT7 plasmid within the cytoplasm, reducing its luciferase expression levels.

The transfection efficiency of lipids 1 and 2 were also compared to that obtained with the commercially available lipids FuGENE™ 6 and DC-Chol/DOPE in the following cell lines: A549, 293T7, HeLa, and HepG2. The investigators found that the luciferase activity after transfection of HepG2 cells with cholesterol/lipid 1 was two orders of magnitude higher than that obtained with FuGENE™ 6 and DC-Chol/DOPE. In contrast, the luciferase activity after transfection of A549, 293T7, and HeLa cells was similar to that obtained using the commercial lipids. Also, the authors found that the transfection efficiency obtained with cholesterol/lipid 1 were superior to that obtained from cholesterol/lipid 2 across a range of cholesterol to lipid molar ratios. However, this relationship was reversed in 293T7 cells, perhaps due to enhanced release of the plasmid and/or better accessibility by T7 RNA polymerase.

### **Membrane Disruption Capacity**

The investigators then examined the membrane-disrupting capacity of the histidylated lipids using the fluorescence resonance energy transfer method developed by Struck, *et al.* (2). In this technique, an energy donor fluorophore transfers energy to an energy acceptor fluorophore when the first is excited and is in close proximity to the second. The energy acceptor fluorophore then fluoresces as though it were excited directly. An increase in distance between the two fluorophores results in a less efficient energy transfer. To utilize this technique for measuring membrane disruption, the investigators prepared a liposome consisting of egg yolk phosphatidylcholine (PC) and the FRET energy donor and acceptor fluorophores, NBD-PE and Rho-PE. Upon mixture with cholesterol/lipid 1 with the PC/NBD-PE/Rho-PE liposome, membrane fusion measurements were taken in the pH range of 5.0 – 7.4 to mimic the typical acidity of the endosomal lumen. At pH 7.4, no significant loss of FRET activity was detected, indicating no liposome disruption. However, when the procedure was repeated at pH 5.0 or 6.0, a significant loss of FRET efficiency was observed. The extent of acid induced loss of FRET activity depended on the amount of cholesterol/lipid 1 added, clearly indicating significant membrane fusion. A similar result was seen when using liposomes made from PC, phosphatidylethanolamine (PE), phosphatidylserine (PS), cholesterol, NBD-PE,

and Rho-PE, a lipid composition similar to that of the plasma membrane. These results suggest that protonation of the histidine head group of lipid 1 in slightly acidic conditions destabilizes lipid bilayers, thereby inducing membrane fusion. This interpretation is supported by the cytosolic transgene expression results previously described for the pT7 luciferase vector.

## Conclusions

The results of this study show that addition of a single histidine residue to a typical cationic lipid amphiphile is sufficient to impart significantly improved transfection properties on the resulting histidylated compound. The improved transfection efficiencies obtained are likely due to the endosome disrupting capacity of the histidine imidazole moiety when protonated under acidic endosomal conditions. The authors indicate that further structure-function studies using a series of monohistidylated cationic lipids with varying hydrophobic chain lengths are worthwhile, and are being undertaken in their laboratories.

## References

1. Kumar, VV, Pichon, C., Refregiers, M., Guerin, B., Midoux, P., Chaudhuri, A. (2003) Single histidine residue in head-group region is sufficient to impart remarkable gene transfection properties to cationic lipids: evidence for histidine-mediated membrane fusion at acidic pH. *Gene Therapy* **10**: 1206-1215.
2. Struck DK, Hoekstra D, Pagano RE. (1981) Use of resonance energy transfer to monitor membrane fusion. *Biochemistry* **20**: 4093–4099.