Cationic Liposomes-Mediated Co-delivery of Plasmid DNA and siRNA for a Synergistic Gene Therapy

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ABSTRACT

The aim of this study was to investigate the optimal complexation ratio of plasmid DNA (pDNA) and siRNA combined with cationic liposomes. The amount of cationic liposomes was varied with a fixed amount of pDNA and siRNA to observe in which proportion they could form the optimal combination. The co-complexation effect of pDNA and siRNA was investigated by gel retardation, mRNA expression and green fluorescence protein (GFP) expression. Complexes between pDNA and cationic liposomes were formed at the ratio of 0.8:1 (w/w), whereas siRNA and cationic liposomes were complexed at ratio of 4:1 (w/w). pDNA and siRNA did not interfere each other in the expression of target gene. Therefore, the optimized co-complexation of pDNA and siRNA could be a win-win strategy for gene therapy.

INTRODUCTION

Recent advances in human genome project, gene therapy is in the limelight of new paradigms for therapeutics. Gene therapy is a technique for correcting defective genes that are responsible for disease development. There are three approaches such as replacing a mutated gene that causes disease with a healthy copy of the gene, inactivating or knocking out a mutated gene that is functioning improperly, and introducing a new gene into the body to help fight a disease. In general, pDNA has been used to replace a gene or to introduce a new gene, whereas siRNA has been applied to inactivate or to knock out an improper gene. Therefore, therapeutic synergism would be expected if pDNA and siRNA are transferred simultaneously by one delivery vehicle.

Many cationic lipid formulations have been tested for in vitro and in vivo transfection of nucleic acid. Among lipid-based gene delivery systems, cationic liposomes have been applicable as a useful non-viral delivery system. Since cationic liposomes form the transfection complexes with negatively charged pDNA or siRNA, co-loading of pDNA and siRNA alters electrostatic complexation, which results in the transfection efficiency and inhibition of RNA or protein expression.

In this study, various ratios of pDNA/siRNA and cationic liposomes were prepared to study the co-delivery of pDNA and siRNA complexed in cationic liposomes. Furthermore, the changes in complexation, mRNA expression, and protein expression were investigated.

EXPERIMENTAL METHODS

Cationic liposomes were prepared by mixing cationic lipid, DOTAP, and neutral lipid, DOPE at 1:1 ratio. As a model pDNA, resistin-encoding pDNA was cloned by inserting resistin in pVAX1 vector (Invitrogen). The siRNA sequences for the targeted silencing of green fluorescence protein (GFP) were used. Various ratios of lipoplexes were prepared by cationic liposomes, pDNA, or siRNA. To confirm the complexation, gel retardation was carried out on electrophoresis and the effect of added pDNA or siRNA on the co-complexation with cationic liposomes was compared.

After the complexation ratio was optimized, in vitro transfection study was performed in H4II-E cells (rat hepatoma cells), stabilized to express green fluorescence protein (GFP). To compare transfection efficiency, GFP intensity was measured by flow cytometry and mRNA expression of resistin was compared by RT-PCR. To visualize the GFP expression in cells,
confocal laser scanning microscope was used with excitation at 488 nm.

RESULTS AND DISCUSSION

Transfection complexes between DNA and cationic liposomes were formed at the ratio of 0.8:1 (w/w), whereas the light band of DNA disappeared at the ratio of 4:1 (w/w) (data not shown). The band of siRNA began to disappear at the amount of pDNA equal to 120 ng. The complexation of pDNA/siRNA and cationic liposomes could be optimal at the ratio of 110:80:350 (w/w/w) in nanogram levels.

As shown in Fig. 1, siRNA did not hamper the mRNA expression of resistin on the co-complexed with cationic liposomes. Moreover, the co-delivery of siRNA increased the mRNA levels compared to pDNA complexed alone with cationic liposomes. Therefore, further investigation on the mechanism of synergism should be planned.

Addition of pDNA did not interrupt the activity of GFP-specific siRNA, showing the inhibition of GFP expression at similar levels with siRNA alone complexed with cationic liposomes (Fig. 2). However, synergistic effect of added pDNA on the inhibition by siRNA was not observed and the reduction of GFP expression was not significantly different (Fig. 3).

CONCLUSION

The complexation of pDNA/siRNA and cationic liposomes could be optimal at the ratio of 110:80:350 (w/w/w) in nanogram levels. pDNA and siRNA did not interfere each other in the expression of target gene. Therefore, the optimized co-complexation of pDNA and siRNA could be a win-win strategy for gene therapy.

REFERENCES

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