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Self-assembling Modified β -cyclodextrin Conjugated siRNA Nanoparticles and Liposomal siRNA Efficiently Knock Down the Mutant Huntingtin Gene in a Modified PC-12 Cell Line

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Purpose

To formulate and characterize liposomal siRNA (LP-siRNA) and cationic β-cyclodextrin siRNA (CD-siRNA) nanoparticle complexes and evaluate their efficacy in knockdown of mutant Huntingtin protein (mHtt) in a modified PC-12 cell line.

Methods

Liposomes were prepared by thin film hydration using molar ratio 50:50 of DOTAP/DOPE. Probe sonication was used to reduce the particle size of liposomes. The surface charge and effect of sonication on particle size were studied using photon correlation spectroscopy. Cationic β-cyclodextrin nanoparticles were synthesized by one-step condensation polymerization using β-cyclodextrin (β-CD), epichlorohydrin (EP) and choline chloride (CC) in molar ratios (1/5/2), (1/15/1) and (1/15/2). Unmodified β-CD monomers were separated by dialysis for 24 h. The β-CD nanoparticles were characterized for surface charge, particle size, percent yield, and structural polymeric substitution by proton nuclear magnetic resonance (1H NMR). The LP-siRNA and CD-siRNA complex formations were confirmed by gel retardation assay using siRNA-HDex1 (5' GCCUUCGAGUCCCUCAAGUCC-3'). MTT assay based cell viability studies were conducted with naked-siRNA, blank CD nanoparticles, blank liposomes, LP-siRNA and β-CD-siRNA complexes using PC-12 cells in which mHtt formation was chemically induced. Huntingtin protein expression was determined by western blot analysis before and after cell treatment with naked siRNA, LP-siRNA and CD-siRNA formulations.

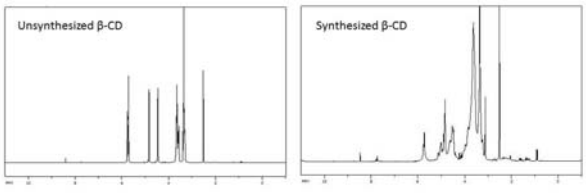
Results

The average particle size of liposomes was 115 nm following 4 min probe sonication, beyond which time no further size reduction was observed. The average surface charge was +28 mV for blank liposomes (LP) and +18.3 mV for liposomal siRNA complexes (LP-siRNA), indicating that complexation reduces the surface charge of the cationic liposomes. For the molar ratios (1/5/2), (1/15/1) and (1/15/2), the average particle size of the nanoparticles were 146.2nm, 184 nm and 158.3nm, respectively, and corresponding surface charge were + 32.6 mV, +26mV and +20.4mV. The practical yield obtained was highest for 1/5/2 (74%), compared to 1/15/1 (51%) and 1/5/2 (44%). Based on surface charge and practical yield, molar ratio 1/5/2 was selected for further experiments. The average surface charge of the synthesized nanoparticles was reduced to +15.2 mV after CD-siRNA complex formation. Proton nuclear magnetic resonance (1H NMR) spectra confirmed the functional polymeric end groups and introduction of cationic groups in the β-CD backbone. Gel retardation assay showed formation of electrostatic complexes of LP-siRNA and CD-siRNA at 10 nM and 20 nM concentrations of siRNA-HDex1. When normalized against the media control, the MTT assay data showed comparable or better cell survival for free siRNA, drug-free liposomes, drug-free CD nanoparticles, LP-siRNA and CD-siRNA nanoparticle complexes, indicating the lack of toxicity for the formulations being studied. Western blot indicated efficient knockdown of huntingtin protein after treatment with LP-siRNA and synthesized β-CD-siRNA nanoparticle complexes.

Conclusion

Liposomal-siRNA (LP-siRNA) and β-CD-siRNA nanoparticle complexes were successfully formulated and shown to be effective in decreasing the levels of mutant huntingtin (mHtt) protein compared to naked siRNA treatment in PC-12 cells.

1H NMR Spectra



Gel Retardation Assay