Lipid modified aminoglycoside based polymers as efficient transgender expression agents

Bhavani Miryala,[a,] Thrimoorthy Potta,[b,] Kaushal Rege [a] *

[a]Chemical Engineering, Arizona State University ,Tempe, AZ 85287.

[b]Marlyn nutraceuticals, Inc, 4404 E. Elwood street, Pheonix, AZ 85040.

Abstract:

Hydrophobic modification of polymers proved to be an efficient method to improve the transfection efficacies of cationic polymers. New lipopolymers were synthesized by conjugating different aliphatic lipids to different aminoglycoside based polymers (polymers already developed in the lab) via *N*-acylation to develop efficient gene delivery systems. The synthesis resulted in a library of lipopolymers which are well characterized using ^{1}H NMR and FT-IR spectroscopy. The lipids used in the synthesis differ by chain length and the polymers used differ in the number of amino groups thereby facilitating the chances of knowing multiple factors affecting the transfection profiles in a single study. The extent of lipid substituted on the polymer varied according to the feed molar ratio. The results from the transfection profiles of lipopolymers evaluated in different types of cancer cells led to identification of some leads which proved to be more efficient in transfection when compared to the parent polymers and the standard poly(ethyleneimine). The toxicity profiles of the parent polymers did not change much on conjugation with lipids.

Materials: The polymers synthesized were dissolved in DMSO(Dimethyl sulfoxide) (2 mL) at room temperature, triethyl amine (in 1:4 molar ratio with respect to polymer) and different amounts of alkanoyl cholorides (1:2, 1:5, 1:10 corresponding to different molar ratios with respect to polymer) were added and the mixture was stirred at room temperature for 12 h. The final product was collected by precipitation in excess ether, dialyzed and freeze-dried. Transfection studies were carried out using different types of cancer cells and using pGL4.5 plasmid. The amount of luciferase expressed (reported gene) was reported in terms of RLU/mg of protein.

Results: A library of lipopolymers were synthesized following the synthetic protocol as shown in Figure. 1a**.** The synthesized polymers are well charcterized. Figure 1b shows the ^{1}H NMR spectra of a representative polymer and its lipid conjugate.The lipopolymers synthesized were tested for in vitro gene transfection studies using pGL4.5 as plasmid vector which encodes for the modified firefly luciferase protein. Figure 1c shows the transfection profiles of some of the leads in Prostrate Cancer cells (PC3) and compared to their parent polymers and standard poly(ehtyleneimine).

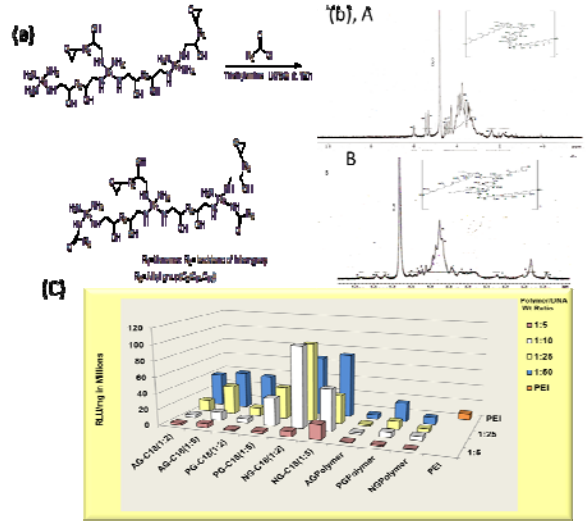

Figure 1. (a) Scheme for synthesis of lipopolymers, (b) 1H NMR spectra of Neomycin-GDE polymer (A) and Neomycin-GDE polymer conjugated with hexanoyl chloride (B), (c) In vitro transfection profile of leads of lipopolymers in PC3 cells and compared with their parent polymers and standard poly(ethylenimine).

Conclusions: A small library of aminoglycoside based lipopolymers using aliphatic lipids were successfully synthesized and well characterized. High throughput screening of lipopolymers to evaluate their transfection profiles showed some interesting leads which showed very high transfection profiles compared to standard. Serum stability results showed that some of the lead lipopolymers are highly serum stable and thus can be used for in vivo studies.

References:

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