Development of gelatin nanocarriers for the targeted delivery of STAT-6 siRNA

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ABSTRACT SUMMARY

A gelatin nanocarrier system was developed to encapsulate and deliver STAT-6 siRNA (S6S) to Th-2 cells to alleviate asthma. This biocompatible gelatin polymer based nanoparticle (GNC) approach provided improved stability and enhanced potency of S6S.

INTRODUCTION

Asthma is a complex disease that requires long-term and multifaceted treatments. Existing treatment options, such as anti-histamines or steroids are nonspecific, fail to prove successful control of one’s symptoms, and often produce undesirable adverse effects1. A recent national asthma survey found that asthma healthcare is suboptimal and that the disease remains poorly controlled2. This prompts a need for a more targeted approach for mitigating atopic, or allergic, asthma processes.

Signal transducer and activator of transcription factor 6 (STAT-6) drives T helper 2 (Th2) allergic asthma and the up-regulation of STAT-6 in the airways are associated with severe cases of asthma3,4. Therefore, it is hypothesized that the targeted delivery of STAT-6 small interfering RNA (S6S) using a parenteral cationic gelatin nanocarrier (GNCa) will selectively down regulate STAT-6 expression and thereby produce Th2 response inhibition and lead to reduced symptoms of allergic asthma. As a result, the proposed studies will have significant impact in the area of effective siRNA delivery for the treatment of asthma.

Nanocarriers (NC) have generated strong attention for siRNA delivery owing to their ability to overcome issues relating to siRNA delivery5,6. An ideal delivery system must be biocompatible, biodegradable, and site-specific, while both avoiding siRNA degradation and evading uptake by normal cells6. Gelatin is a biodegradable and biocompatible natural polymer that is approved by the FDA for IV administration. These benefits, along with non-toxicity, ease of chemical modifications and cross-linking capacities, make it a desirable non-viral vector.

The goals of this study are to formulate S6S loaded GNC (S6S-GNCa) and evaluate in-vitro STAT-6 silencing by the STAT-6 siRNA-GNC and downstream marker expression. The long term objective of this project is to develop S6S-GNC for the effective treatment of asthma.

EXPERIMENTAL METHODS

Stat-6 loaded gelatin nanocarriers (S6S-GNCa) was prepared by the two-step desolvation method employing 3^3 factorial design using different dependent and independent variables. Dependent variables were particle size, zeta potential, and entrapment efficiency while concentration of gelatin (0.5, 1, 1.5 %w/v), total volume of non-solvent (70, 80, 90 %v/v) and genipin crosslinking amount (0.2, 0.7, 1.0 % weight genipin to gelatin) were evaluated as independent variables. An example preparation is as follows: The high-molecular weight fraction was prepared by the classical 2-step desolvolation technique, where 5% (w/v) gelatin type “A” was first desolvated with an equal volume of acetone for 12 minutes under gentle stirring. After 12 minutes, the supernatant, which contained the low molecular weight gelatin fraction, water, and acetone was decanted and discarded. The high-molecular weight fraction sediment was allowed to dry and underwent mass reconciliation. Using the mass, a certain amount of water was added to re-dissolve the HMW gelatin to a 1% (w/v) solution at 50°C under gentle stirring. When the gelatin solution became homogeneous and transparent, the temperature of the solution was reduced to 35°C and STAT-6 siRNA was added and allowed to incubate for 10 min with gentle stirring. A second desolvation step commenced, where ethanol was added drop wise at a rate of 1 ml/min under a constant stirring rate of 600 rpm. Two minutes after the ethanol addition ended, varying levels of genipin was added drop wise at a rate of 0.2 ml/min to crosslink the nanocarriers. The formulation was stirred at a rate of 600 rpm for another 55 min and then 5 ml ddi H2O was added and the stir rate was reduced to 200 rpm until ethanol completely evaporated. In total, 27 S6S-GNC batches were prepared.

The nanoformulation was characterized exhaustively for loading efficiency, size, charge, release kinetics, stability and cytotoxicity performance. The size and charge of S6S-GNC was measured using NICOMP 380 ZLS, USA. Entrapment efficiency was determined by measuring free STAT-6 siRNA using RNA analysis method (BioSpec Nano, USA). In vitro release studies were performed using cellulose membrane (MW cut off 50 KDa) and PBS (pH 7.4) as dissolution medium to evaluate release pattern. The stability of S6S-GNC was assessed under conditions of varying pH and serum level to determine the stability of developed formulation. The S6S-GNC was incubated in a phosphate buffer (pH 6.4, 7.4 and 8.4) to assess the influence of pH on charge and size of the GNC. Additionally, the stability was also accessed using agarose gel electrophoretic mobility assay. The in-vitro cytotoxicity of the optimized S6S-GNC and appropriate controls was evaluated against H460 and A549 lung cancer cells following MTT cytotoxicity assay.
RESULTS AND DISCUSSION

The hydrophilic nature of anionic S6S and cationic gelatin will help to achieve electrostatic interaction and the desired incorporation of the S6S within the GNC. The developed formulation contained ≈10,000 GNC/ml, and the average particle size and surface charge was observed to be 69.6±6.5 nm and +10±1.5 mV, respectively. The optimized S6S-GNC formulations showed an encapsulation efficiency of 85±4%. S6S-GNC showed an insignificant (p<0.05) change in the size and charge in the presence of buffer solutions (pH 6.4 to 8.4) and FBS (10% v/v) as shown in Figure 1. In-vitro dissolution testing of the S6S-GNC exhibited a controlled release.

A549 cells were treated with native S6S, S6S-lipofectamine, placebo GNC and S6S-GNC using untreated cells as a control. It was observed that % cell viability was decreased significantly with S6S-GNC by 55±4 % (p<0.001) compared to native S6S (2±0.5%) and S6S-lipofectamine (40±3 %), as seen in Figure 2. Placebo GNC treatment showed >97% viability of cells demonstrating non-toxicity and safety of GNC formulation.

CONCLUSION

A STAT-6 siRNA loaded gelatin nanocarrier was developed using the 2-step desolvation technique and genipin as a crosslinker. Our results demonstrate the formulation of stable and function siRNA loaded superior GNC (S6S-GNC). Hence, the utility of the gelatin nanocarrier approach for enhanced therapeutic siRNA delivery was confirmed from this study. Therefore, the STAT-6 siRNA delivery via the gelatin nanocarrier delivery system has the potential for improving targeting and minimizing off-target effects, especially through the use of surface modification, such as targeting ligands. We are further investigating the efficacy of S6S-GNC on gene silencing and protein expression against mouse T lymphocytes and T helper 2 cells.

REFERENCES


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