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An albumin-mediated cholesterol design-based strategy for tuning siRNA pharmacokinetics and gene silencing

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Abstract

Major challenges for the clinical translation of small interfering RNA (siRNA) include overcoming the poor plasma half-life, site-specific delivery and modulation of gene silencing. In this work, we exploit the intrinsic transport properties of human serum albumin to tune the blood circulatory half-life, hepatic accumulation and gene silencing based on the number of siRNA cholesteryl modifications. We demonstrate by gel shift assays a strong and specific affinity of recombinant human serum albumin (rHSA) towards cholesteryl-modified siRNA (K_d > 1 x 10^{-7} M) dependent on number of modifications. The rHSA/siRNA complex exhibited reduced nuclease degradation detected by a gel shift assay and reduced induction of TNFα production by human peripheral blood mononuclear cells. The increased solubility of heavily cholesteryl modified siRNA in the presence of rHSA facilitated duplex annealing and consequent interaction that allowed *in vivo* studies using multiple cholesteryl modifications. A structural-activity-based screen of *in vitro* EGFP-silencing was used to select optimal siRNA designs containing cholesteryl modifications within the sense strand that were used for *in vivo* studies. We demonstrate plasma half-life extension in NMRI mice from $t_{½}$ 12 min (naked) to $t_{½}$ 45 min (single cholesteryl) and $t_{½}$ 71 min (double cholesteryl) using fluorescent live bioimaging. The biodistribution showed increased accumulation in the liver for the double cholesteryl modified siRNA that correlated with an increase in hepatic Factor VII gene silencing of 28% (rHSA/siRNA) compared to 4% (naked siRNA) 6 days post-injection. This work presents a novel albumin-mediated cholesteryl design-based strategy for tuning pharmacokinetics and systemic gene silencing.

1. Introduction

RNA interference (RNAi) is a conserved biological process in which double stranded RNA including small interfering RNA (siRNA) mediates sequence-specific degradation of target mRNA by the RNA-induced silencing complex (RISC) [1, 2]. RNAi-mediated silencing of disease-associated genes offers great therapeutic potential; however, clinical translation of RNAi-based therapeutics has not yet been realized.

Major challenges such as serum degradation, rapid renal clearance, non-specific accumulation and poor cellular uptake restrict the *in vivo* effectiveness. RNAi therapeutics, therefore, require enabling technologies such as chemical modification and delivery science to overcome extracellular and intracellular barriers. Modifications such as 2′-O-methyl RNA (2′O-Me), 2′-O-methoxyethyl (2′MOE), locked nucleic acids (LNA) and 2′-fluoro (2′F) have been widely used to increase stability and potency of the RNAi therapeutics [3, 4]. In addition lipid- or polymer-based nanoparticles have been used to