Development of targeted siRNA nanotherapeutics to prevent fibrosis in experimental glaucoma filtration surgery

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Abstract

Purpose: RNA interference is a promising therapeutic approach as it can be used to silence the expression of pathogenic genes in a wide range of diseases. We hypothesised that siRNA nanoparticles against the MRTF-B gene could be used as a targeted anti-fibrotic therapy in glaucoma filtration surgery.

Methods: We tested the effect of 15 liposome-peptide-siRNA nanoparticle formulations on MRTF-B gene silencing using real-time quantitative PCR and on cell viability in human conjunctival fibroblasts. Nanoparticle size, zeta potential and morphology were determined by dynamic light scattering, laser Doppler anemometry and transmission electron microscopy, respectively. We validated our results using a randomised, prospective masked, crossover study of 18 New Zealand white female rabbits undergoing glaucoma filtration surgery. The animals received intraocularly 0.2 mg/mL mitomycin C (MMC) [N=6], or a postoperative subconjunctival injection of 25 μg MRTF-B nanoparticles [N=6] or control nanoparticles [N=6]. Bleb morphology was recorded over 30 days. Tissue sections on day 30 were immunohistologically assessed. We analysed our results using Kaplan-Meier curve, Log-rank test and Student's t-test.

Results: in vitro, targeted LYR [OCT1/UCP2-peptide siRNA] formulation was the most efficient (52.7% MRTF-B gene silencing) and non-cytotoxic compared to the non-targeting peptide formulation (23.7% silencing). LYR nanoparticles were spherical particles of 108.3 ± 6.4 (SEM) nm, 96.2 ± 7.1 nm and 0.37 ± 0.01 polydispersity index, Anion-exchange nanoparticles had no effect on the MRTF-B gene. In vivo, bleb survival was increased from 1.0 ± 3.0 (SEM) days for control nanoparticles to 22.0 ± 2.1 days for MRTF-B LYR nanoparticles (p=0.003) and 27.5 ± 1.3 days for MMC (p=0.001). MRTF-B LYR nanoparticles and MMC reduced conjunctival scarring compared to control nanoparticles, was stained by H&E, a cell red, Gomori's chromo and alpha smooth muscle actin staining. MRTF-B LYR nanoparticles were also not toxic and silenced the MRTF-B gene by 25.6% (p=0.046), in rabbit conjunctival tissues.

Conclusions: Receptor-targeted liposome-peptide-siRNA nanoparticles represent a safe and efficient siRNA delivery system that could be used to prolong bleb survival and to prevent conjunctival fibrosis after glaucoma filtration surgery by targeting the MRTF-B gene and potentially other gene targets associated with fibrosis.

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