siRNA Silencing of Gene Expression in Trabecular Meshwork: RhoA siRNA Reduces IOP in Mice

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Few reports described efficient transfection in the trabecular meshwork (TM) in vivo. In the present study, we investigated the distribution of cy3-labeled siRNAs after of wing injection into the anterior chamber (AC) and explored the use of RhoA siRNA (siRhoA) to modulate intraocular pressure (IOP) through down-regulation of RhoA gene and protein expression.

Cy3-labeled siRNAs were injected into the AC to investigate the distribution. In addition, siRhoA was applied to normal and DEX-induced elevated IOP mice. The RhoA gene was detected at 1d post-injection (PI) using real-time RT-PCR. Proteins were examined using immunofluorescence staining at 1, 2, and 3 day PI. IOP was measured pre- and post-injection using a TONO-PEN.

Toxicity was preliminarily assessed using clinical observation and hematoxylin-eosin staining. The study demonstrated that cy3-labeled siRNAs accumulated in mouse TM in a dose-dependent manner, with a peak at 24h PI. There was no visible siRNA fluorescence in the corneal endothelium, and little in the iris. siRhoA caused large decreases in RhoA mRNA and protein expression in mouse TM (p<0.01).

In normal mice, injections of siRhoA induced decreases in IOP, by 2d, with recovery to baseline by 3d PI. For DEX-treated animals, IOP significantly decreased from 2d to 5d PI (p<0.05). There was no obvious toxicity after the siRhoA application. These results suggest that (1) siRNA injection into the AC leads to transient gene transfection in TM; (2) inhibiting RhoA expression in TM with siRNA is effective in suppressing elevated IOP in mice, suggesting that siRhoA is a potential pharmaceutical intervention for glaucoma.

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