Suprachoroidally delivered non-viral DNA nanoparticles produce hMyo7A Protein in RPE-choroid in rabbits

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Purpose

- Suprachoroidal (SC) delivery offers the potential to target chorioretina while avoiding surgical risks associated with a subretinal injection and may offer a novel alternative for office-based gene therapy for the treatment of inherited retinal diseases[1].
- Non-viral based gene delivery offers the potential for repeatable (if needed) gene therapy and affords delivery of large size plasmid (>10kb) that cannot be accommodated by a single AAV vector[2].
- Functional deficiency of Myosin (Myo) 7A protein is implicated in the pathogenesis of Ushers syndrome[3], an inherited retinal disease and a form of retinitis pigmentosa.
- The purpose of this research was to evaluate ocular tolerability and chorioretinal cell transfectability of SC and intravitreal (IVT) injected non-viral DNA nanoparticles (DNPs) containing plasmid encoded for hMyo7A.

Methods

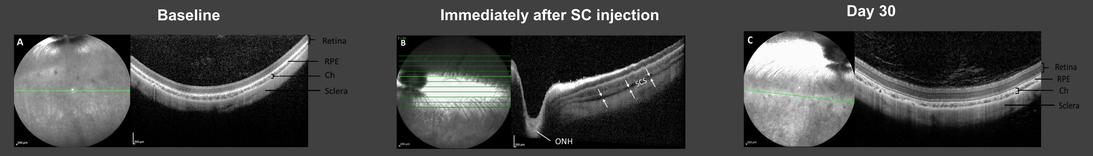
- Dutch-Belted pigmented rabbits (N=4 eyes per group) received a single SC (0.1 mL) or IVT injection (0.05 mL) of DNPs (either 4 or 8 mg/mL of DNA).
- The DNPs consisted of a single copy of plasmid DNA encoding hMyo7A with a polyubiquitin C transcriptional cassette.
- DNP-1 consist of pDNA encoding natural hMyo7A, DNP-2 consist of pDNA encoding codon optimized hMyo7A.
- Ocular tolerability was assessed via slit lamp, indirect ophthalmoscopy, intraocular pressure (IOP), optical coherent tomography (OCT), electroretinography (ERG) and fundus photography (FP) for up to 3 months.
- Protein (hMyo7A) and RNA levels were measured in ocular tissues via ELISA and qRT-PCR, respectively, at 3 months.
- Expression pattern of eGFP was assessed longitudinally via in-vivo imaging method

Group	Test article	Injection (route and volume)	Concentration
1	DNP-1	SC, 100 µL	4.4 mg/mL
2		IVT, 50 µL	4.4 mg/mL
3	DNP-2	SC, 100 µL	4 mg/mL
4		IVT, 50 µL	4 mg/mL
5	DNP-1	SC, 100 µL	8.3 mg/mL
6	DNP-2	SC, 100 µL	8 mg/mL
7	DNP-1, eGFP	SC, 100 µL	4.2 mg/mL
8	DNP-1, eGFP	IVT, 50 µL	4.2 mg/mL

Results

1 SC injection resulted in reversible opening of suprachoroidal space

Representative optical coherent tomography images of rabbit eyes

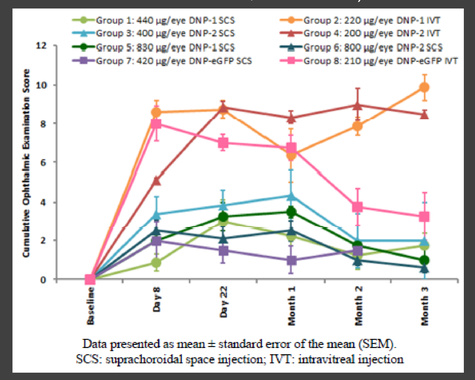


Ocular Tolerability

2 Suprachoroidally injected DNPs exhibited better ocular tolerability with milder and less frequent ocular findings compared to IVT injected DNPs

Cumulative ocular exam scores

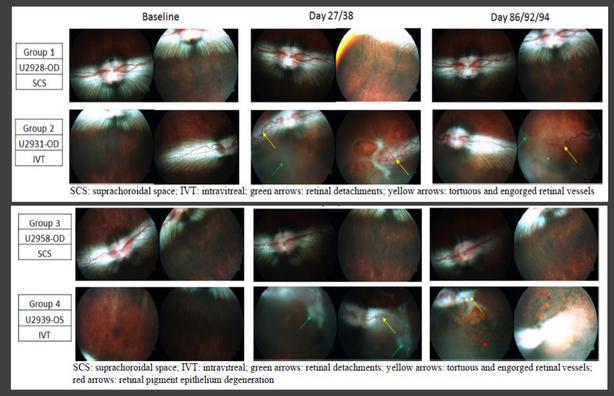
(0-2: minimal; >2-4: mild; >4-6: moderate; >6-8: marked; >8: severe)



Data presented as mean ± standard error of the mean (SEM).
SCS: suprachoroidal space injection; IVT: intravitreal injection

Fundus Photographs

(representative images)



SCS: suprachoroidal space; IVT: intravitreal; green arrows: retinal detachments; yellow arrows: tortuous and engorged retinal vessels

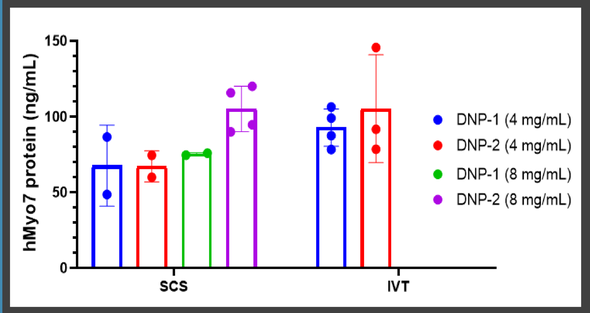
SCS: suprachoroidal space; IVT: intravitreal; green arrows: retinal detachments; yellow arrows: tortuous and engorged retinal vessels; red arrows: retinal pigment epithelium degeneration

In-Vivo Transfection (hMyo7A Protein and mRNA Levels)

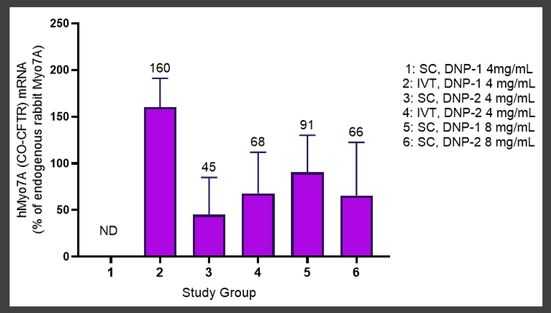
3

✓ **SC and IVT injected DNPs produced hMyo7A protein in the RPE-choroid**
✓ **Protein and mRNA levels were near or below level of detection in retina**

RPE-choroid
(hMyo7A protein via ELISA)



RPE-choroid (mRNA via RT-PCR)
hMyo7A as % of endogenous rabbit Myo7A



ND: not detected; CO-CFTR in 3'-UTR (untranslated region)

Key Findings

- ✓ Suprachoroidally injected non-viral DNPs were generally well-tolerated in rabbits.
- ✓ IVT injected DNPs exhibited higher incidence and degree of intraocular inflammation, lens opacity, and retinal detachment/ degeneration.
- ✓ Acute increase in intraocular pressure was observed after either SC or IVT injection of DNPs which returned to the baseline level at the next assessment timepoint (month 1).
- ✓ OCT imaging confirmed reversible opening of SC space immediately after the SC injection.
- ✓ The hMyo7A protein was detected in the RPE-choroid (68-105 ng/gm) at 3 months after SC or IVT injection with no statistically significant difference between the routes of administration.
- ✓ The hMyo7A protein levels were detected in sporadic retina samples.
- ✓ The RT-PCR data indicate that DNPs produce mRNA levels in the RPE-choroid that is in the range of 45%-160% of the endogenous rabbit Myo7A.

Conclusions

- ❖ SC DNPs containing transgene encoded for hMyo7A produced efficient and durable levels of hMyo7A in RPE-choroid.
- ❖ Photoreceptor (PR) specific promoters will be assessed to transfection in PR in future studies.
- ❖ The immune response to human Myo7A in rabbits may have impacted the levels observed in this study.
- ❖ Further studies in clinically relevant higher species (monkeys) are warranted.
- ❖ SC non-viral DNP-based gene delivery has potential as an office-based repeatable therapy for large-gene disorders.

References

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