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Structural recovery of the retina in a retinoschisin-deficient mouse after gene replacement therapy by solid lipid nanoparticles

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ABSTRACT

X-linked juvenile retinoschisis (XLRS) is a retinal degenerative disorder caused by mutations in the *RS1* gene encoding a protein termed retinoschisin. The disease is an excellent candidate for gene replacement therapy as the majority of mutations have been shown to lead to a complete deficiency of the secreted protein in the retinal structures. In this work, we have studied the ability of non-viral vectors based on solid lipid nanoparticles (SLN) to induce the expression of retinoschisin in photoreceptors (PR) after intravitreal administration to Rs1h-deficient mice. We designed two vectors prepared with SLN, protamine, and dextran (DX) or hyaluronic acid (HA), bearing a plasmid containing the human *RS1* gene under the control of the murin opsin promoter (mOPS). In vitro, the nanocarriers were able to induce the expression of retinoschisin in a PR cell line. After injection into the murine vitreous, the formulation prepared with HA induced a higher transfection level in PR than the formulation prepared with DX. Moreover, the level of retinoschisin in the inner nuclear layer (INL), where bipolar cells are located, was also higher. Two weeks after vitreal administration into Rs1h-deficient mice, both formulations showed significant improvement of the retinal structure by inducing a decrease of cavities and PR loss, and an increase of retinal and outer nuclear layer (ONL) thickness. HA-SLN resulted in a significant higher increase in the thickness of both retina and ONL, which can be explained by the higher transfection level of PR. In conclusion, we have shown the structural improvement of the retina of Rs1h-deficient mice with PR specific expression of the *RS1* gene driven by the specific promoter mOPS, after successful delivery *via* SLN-based non-viral vectors.

1. Introduction

X-linked juvenile retinoschisis (XLRS) is the leading cause of macular degeneration in males, with an estimated prevalence ranging from 1 in 15,000 to 1 in 30,000. This amounts to approximately 5% of the childhood-onset inherited retinal dystrophies [1]. XLRS is associated with mutations in the *RS1* gene, which encodes a protein termed retinoschisin. This discoidin-domain containing protein binds mainly to the surface of photoreceptors (PR) and bipolar cells where it appears to maintain the cellular organization of the retina and the structure of the PR-bipolar synapse [2]. XLRS patients suffer from mild to severe loss in visual acuity, splitting of retinal layers owing to cystic cavities formation, and loss of PR leading to a reduction in the electroretinographic b-wave. The worst outcome in visual acuity is seen in those patients with extensive schisis that involve the central retina. Vision in most affected males is usually relatively stable until adulthood, when progressive macular atrophy may develop [3]. Currently, there is no cure for the schisis formation, and the treatment is limited to the preservation and prevention of the vision loss. Due to the lack of an effective treatment and because the underlying deleterious effects of the known disease mutations are well understood, XLRS is an excellent candidate for gene replacement therapy.

Such a treatment option has been successfully tested in animal models of XLRS *via* viral vectors. The delivery of a normal copy of

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the *RS1* gene using this kind of vectors was shown to ameliorate degeneration [4,5]. Non-viral vectors were also investigated as carriers of genetic material to the eye [6,7]. Lipid-based colloidal vectors, and more specifically solid lipid nanoparticles (SLNs), are considered as one of the most effective non-viral vectors [8,9], and they represent a promising strategy for the treatment of retinal diseases [10–13].

In a previous study, we developed an SLN-based nanocarrier bearing a plasmid construct with the *RS1* gene under a ubiquitous promoter. We evaluated this construct *in vivo* in an Rs1h-deficient mouse model of XLRS [7]. The vectors, prepared with SLNs, protamine (P) and dextran (DX) or hyaluronic acid (HA) induced the expression of retinoschisin in the retina after injection *via* an intravitreal or subretinal route. Moreover, the vectors were able to partially restore the retina of the retinoschisin-deficient mice, which was related to the production of recombinant retinoschisin [7]. In the mentioned study, gene expression was observed in multiple cell types of the retina, even those that do not endogenously express retinoschisin in wild-type animals, such as ganglion cells (GC). In the present work, a new plasmid was constructed to target heterologous gene expression to PR, the main target cells for XLRS [14]. The murine opsin promoter (mOPS), which exhibits strong specific activity in PR, specifically rod cells [15], was included to drive the expression of retinoschisin. As control, this plasmid also encoded the green fluorescent protein (GFP) under the control of the cytomegalovirus (CMV) promoter, allowing following the transfection efficiency of different retinal cell types by the nanoparticles. Here, we have studied the ability of the vectors based on SLN bearing the new plasmid to induce the expression of retinoschisin in PR after intravitreal administration to Rs1h-deficient mice. The ability of the vectors to reach the various retinal layers as well as the biodistribution of expressed retinoschisin was also studied.

2. Materials and methods

2.1. Material

Precirol® ATO5 (Gattefossé), 1,2-Dioleoyil-3-Trimethylammonium-Propane Chloride Salt (DOTAP) (Avanti Polar Lipids, Inc., Alabama, USA) and Tween 80 (Panreac, Barcelona, Spain) were used for the SLNs preparation. DNase I (deoxyribonuclease I) was obtained from Sigma Aldrich (Madrid, Spain). DX (Mw of 3.26 KDa) and HA (Mw of 150 KDa) were obtained from Sigma–Aldrich (Madrid, Spain). Mouse polyclonal Anti-RS1, mouse polyclonal anti-Rhodopsin, mouse monoclonal anti-RPE65, rat monoclonal anti-CD44 and AlexaFluor 647-conjugated goat anti-rat IgG were purchased from Abcam (Cambridge, UK). Rabbit polyclonal anti-GFP, AlexaFluor 488-conjugated goat anti-rabbit, AlexaFluor 647-conjugated goat anti-mouse and AlexaFluor 488-conjugated goat anti-mouse were purchased from Life Technologies (Madrid, Spain). Dulbecco's Modified Eagle's Medium (D-MEM), fetal bovine serum and penicillin-streptomycin were obtained from Life Technologies (Madrid, Spain). Reporter Lysis Buffer was obtained from Promega Biotech Ibérica (Madrid, Spain). Micro BCA™ protein assay kit was purchased from Thermo-Scientific Scientific Inc. (Madrid, Spain). Enzyme-linked Immunosorbent Assay (ELISA) Kit for the detection of retinoschisin was obtained from USCN Life Science Inc. (Houston, USA). Gel Red™ was obtained from Biotium (California, USA). Tissue-Tek® O.C.T™ Compound was purchased from Sakura Finetek Europe (Leiden, The Netherlands). Other chemicals, if not specified, were reagent grade from Sigma–Aldrich (Madrid, Spain) and Panreac (Barcelona, Spain). The murine PR derived cell line (661W) used in the present study was provided by Dr Muayyad Al-Ubaidi (University of Oklahoma, Norman, USA).

2.2. Construction of the plasmid pCMV-GFP_mOPS-RS1

The GFP open reading frame (ORF) was ligated into the multiple cloning site of the pcDNA3 (CMV) vector *via* the Kpnl and Notl restriction sites [16]. The pTR-mOPS-GFP vector was kindly provided by William W. Hauswirth (University of Florida, Department of Ophthalmology) and digested with Xba l and Sal ll-extracting GFP and a neomycin expression cassette. The human *RS1* ORF was inserted *via* Xba I and SalI positioning *RS1* between the mOPS-promoter and a BGH polyadenylation site. This *RS1* expression unit consisting of a mOPS-promoter, the human *RS1* ORF, and a BGH polyadenylation site, was extracted by short time digestion with Bgl II. The 1495 bp fragment was inserted into de Bgl II-digested CMV-GFP vector.

By Sanger sequencing, all elements in the final vector were confirmed to be present in the correct quantity and orientation and resulting in a CMV-GFP_mOPS-RS1 plasmid, which encodes both GFP and retinoschisin.

2.3. Preparation of DX-SLN and HA-SLN vectors

SLNs composed of a lipidic core of Precirol® ATO5 and a lipidic surface based on DOTAP and the surfactant Tween 80, were prepared by a solvent emulsification-evaporation technique previously described by del Pozo-Rodríguez et al. [17]. The final vectors were prepared by first forming a complex with P, DX or HA. The complex was then placed in contact with a suspension of previously prepared SLNs, and the electrostatic interactions between the complex and SLNs led to the formation of the vector at a final weight ratio of 1:2:1:5 (DX-P-DNA-SLN) and 0.5:2:1:2 (HA-P-DNA-SLN).

2.4. Characterization of the vectors

2.4.1. Size and zeta potential measurements

Particle size of SLNs, DX-SLN and HA-SLN vectors were determined by photon correlation spectroscopy (PCS). Zeta potentials were measured by Laser Doppler Velocimetry (LDV). Both measurements were performed on a Zetasizer Nano series-Nano ZS (Malvern Instruments, Worcestershire, UK). All samples were diluted in Milli-Q™ water.

2.4.2. DNA binding, enzyme-protection and SDS induced release

The capacity of the vectors to bind the DNA, to protect it from DNase I, and to release it was studied by using an agarose gel electrophoresis assay. Bands were stained with Gel Red™ as previously described [7]. For the protection study, deoxyribonuclease I (DNase I) was added to the vectors in a ratio of 1 U DNase I/2.5 μg of DNA, and the mixtures were incubated at 37 °C for 30 min. Afterwards, 1% sodium dodecyl sulfate (SDS) solution was added to release the DNA from the SLNs; the vectors were also treated only with SDS to assess the ability of the DNA to be released from the vectors.

2.5. In vitro studies

2.5.1. Cell culture

Transfection and cell uptake assays were performed in the 661W cell line [18,19]. Cells were cultured in Dulbecco's Modified Eagle's Medium (D-MEM) high glucose supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin (10,000 U/mL) antibiotic solution. Cells were incubated at 37 °C in 5% CO_2 atmosphere.