Span nanoparticles as more efficient vectors for *in vivo* transfection of photoreceptors and RPE cells than adeno-associated viral vectors

A. Pensado\(^1\), B. Seijo\(^1,2\), B. De La Cerda\(^3\), L. Valdés-Sánchez\(^3\), SS. Bhattacharya\(^3\), A. Sánchez\(^1,2\), FJ. Diaz-Corrales\(^3\).

\(^1\)Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Santiago de Compostela, Campus Vida, 15782 Santiago de Compostela, Spain.
\(^2\)Molecular Image Group, Health Research Institute-University Clinical Hospital of Santiago de Compostela (IDIS), A Choupana 15706 Santiago de Compostela, Spain.
\(^3\)Department of Cell Therapy and Regenerative Medicine, Andalusian Molecular Biology and Regenerative Medicine Centre (CABIMER), Américo Vespucio Ave., 41092 Seville, Spain.

andreapensadolopez@gmail.com

ABSTRACT SUMMARY

Novel nanosystems have been designed for gene therapy using a sorbitan ester as main component. Their *in vivo* evaluation has shown that these nanoparticles are more efficient gene vectors than adeno-associated viral vectors. In addition, their negative surface charge and the safety and low-cost of their components provide an added value over current non-viral vectors for clinical application.

INTRODUCTION

To date, around 247 retinal disease-causing genes and loci have been identified (1). During the last decade, gene therapy is emerging as a promising therapeutic alternative for the treatment of several ocular pathologies including retinal dystrophies (2). However, several barriers to develop clinically viable retinal gene therapies exist. The human retina is composed primarily of highly differentiated neuronal cells which are post-mitotic from birth. Gene therapy with viral vectors has been proved to be an efficient treatment for some retina related diseases, but non-viral vectors are being extensively studied due to the reduction of risks such as oncogenicity or immunogenicity and due to their easier and cheaper manufacture (3).

The objective of this work was to develop two types of nanosystems based on a sorbitan ester (span 80) (SP) and to evaluate their physicochemical properties and *in vivo* transfection capacity after sub-retinal injection as potential new gene delivery systems compared to an adeno-associated viral vector. Concretely, these nanoparticles (NPs) also include in their composition oleylamine (OA) (SP-OA) or poly-L-arginine (PA) (SP-PA).

EXPERIMENTAL METHODS

Nanoparticles preparation

For the elaboration of the nanoparticles, a solution of sorbitan monooleate (Span® 80) in ethanol (organic phase) was prepared and it was subsequently added under magnetic stirring to an aqueous phase, thereby leading to the spontaneous NPs formation (4, 5). It was possible to incorporate other components, such as OA or PA, by dissolving them in the organic or aqueous phases, respectively. The ethanol is finally removed under reduced pressure on a rotary evaporator. The genetic material was associated by incubation with the NPs under stirring at room temperature. For this purpose a model plasmid (GFP) was selected.

Characterization of nanoparticles

Measurements of nanoparticles size and zeta potential were performed by photon correlation spectroscopy and laser Doppler anemometry, respectively. The association of the pDNA to the NPs was determined by gel electrophoresis assays (1% agarose).

- Animals

All experiments described in this work have been performed in compliance with the Spanish and European Union laws on animal care in experimentation. C57 BL/6Ncrl female mice (one month old) were obtained from Charles River Laboratories España. Mice were housed on the Biological Resources Unit of CABIMER and they were kept in a temperature-controlled environment (21 ± 1 °C), relative humidity of 55 ± 5%, light/dark cycle 08h00-20h00 and given standard mouse chow and water *ad libitum*.

- *In vivo* transfection experiments

Adeno-associated viral vectors serotype 2 (AAV2) carrying the GFP gene or SP-PA-GFP or SP-OA-GFP nanoparticles were injected into the sub-retinal space. A 10 μl syringe (Hamilton, Switzerland) with a 33-gauge needle attached to an ultramicropump (World Precision Instruments, Sarasota, FL, USA) was used to slowly inject 1 μl of nanoparticles or AAV2 constructs. PBS or 5% glucose were also sub-retinal injected and they were used as controls. GFP signal were evaluated using a mouse imaging system (Micron III).

RESULTS AND DISCUSSION

We developed SP-OA and SP-PA particles with nanometric size (180 nm and 230 nm, respectively) and
with positive superficial charge (+43 mV and +32 mV, respectively) (Table 1). As it was expected, the superficial charge of NPs change from positive to negative when NPs were incubated with the GFP plasmid due to its inherent negative charge. These results indicate a pDNA effective association (0.2 mg/ml), that was also demonstrated by agarose gel electrophoresis, since there was not detected the characteristic signal bands of free DNA.

<table>
<thead>
<tr>
<th></th>
<th>Size (nm)</th>
<th>PdI</th>
<th>ζ Potential (mV)</th>
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<tbody>
<tr>
<td>SP-OA</td>
<td>179.2±4.9</td>
<td>0.075</td>
<td>+43.5±0.8</td>
</tr>
<tr>
<td>SP-PA</td>
<td>228.1±3.2</td>
<td>0.038</td>
<td>+32.5±1.1</td>
</tr>
<tr>
<td>SP-OA+GFP</td>
<td>340.1±3.6</td>
<td>0.133</td>
<td>-8.3±0.3</td>
</tr>
<tr>
<td>SP-PA+GFP</td>
<td>355.1±1.8</td>
<td>0.067</td>
<td>-7.4±0.2</td>
</tr>
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</table>

Table 1. Physico-chemical characteristics of the NPs.

Figure 1A shows the results obtained after in vivo evaluation. One month after injection the fundus (A-E) and the GFP signal were evaluated (F-J). Eye samples were collected for immunofluorescence studies (K-Y). The GFP signal itself (K-O) or enhanced with anti-GFP antibodies (P-T) were evaluated. The right panel represents the merged images with the cell nuclei stained with DAPI dye (U-Y; blue). PBS (A, F, K, P, U) and 5% glucose (B, G, L, Q, V) injected mice did not show neither fundus abnormalities nor any fluorescence signal in the retina layers. Fundus evaluation of AAV2-GFP (C, H) SP-PA-GFP (D, I) and SP-OA-GFP (E, J) treated mice showed a scar in the area of the sub-retinal injections, and GFP signal was detected over the same injected areas. Note the intense fluorescence signal observed in the SP-OA-GFP injected eye (J). Retinal sections of AAV2-GFP (M, R, W), SP-PA-GFP (N, S, X) and SP-OA-GFP (O, T, Y) treated mice displayed GFP signal in both photoreceptors and RPE cells. Visualization of GFP expression was higher in the SP-OA-GFP injected group (O, T), but the number of nuclei in the ONL were reduced or even were completely absent in the same injected area where the fluorescence signal of GFP was highly detected (Y). This toxic effect may be due to the high expression of GFP produced by the plasmid (SP-OA-GFP), because mice injected with SP-OA without GFP did not show any changes in the retinal layers. Thus, SP-OA nanoparticles are more effective than the other vectors administered at the same dose to transfect photoreceptors and RPE cells, thus changing the current paradigm which relates NPs positive superficial charge with effective transfection.

CONCLUSIONS

A novel nanosystem has been developed that provides higher, safer and cheaper in vivo transfection in the retinal pigment epithelial cells than adeno-associated viral vectors and, therefore, that offers a high clinical potential in the treatment of retinal diseases.

REFERENCES


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