rescifar REVISTA ESPAÑOLA DE CIENCIAS FARMACÉUTICAS

Functionalised SLNs-based nanovectors for gene therapy in Fabry disease: The liver as an α-Galactosidase A factory

Rodríguez Castejón Julen[1,2], Gómez Aguado Itziar[1,2], Beraza Millor Marina[1,2], Solinís María Ángeles[1,2], del Pozo Rodríguez Ana[1,2], Rodríguez Gascón Alicia *[1,2]

[1] Pharmacokinetics, Nanotechnology & Gene Therapy Group (PharmaNanoGene), Faculty of Pharmacy, Centro de Investigación Lascaray Ikergunea, University of the Basque Country UPV/EHU, Paseo de la Universidad 7, 01006 Vitoria-Gasteiz, Spain

[2] Bioaraba, Microbiology, Infectious Disease, Antimicrobial Agents and Gene Therapy, 01006 Vitoria-Gasteiz, Spain

*Correspondencia: alicia.rodriguez@ehu.eus

1. Introduction

Fabry disease (FD) is a monogenic X-linked metabolic disorder caused by mutations in the gene that encodes the enzyme α-Galactosidase A (α-Gal A). A deficiency of enzyme activity leads to a progressive deposition of glycosphingolipids within the lysosomes of cells, predominantly in vascular endothelial and smooth muscle cells [1].

The liver is a highly specialized organ in protein synthesis, which, after transfection with the appropriate nucleic acid, could act as an α-Gal A production factory to later release it and restore the enzyme deficiency in affected organs. The delivery of nucleic acids to hepatocytes with non-viral vectors is challenging; however, it can be enhanced by functionalizing the carriers with different ligands [2].

Solid Lipid Nanoparticles (SLNs) are regarded as one of the most promising non-viral gene delivery systems. One of their main advantages is the wide versatility they offer. In fact, SLNs can be decorated easily with polysaccharides to control the biodistribution in vivo [3].

The objective of the present work is the design of SLNs-based nanovectors decorated with polysaccharides and the evaluation of their capacity to transfect a liver-derived cell line (Hep G2).

2. Materials and methods

2.1. Preparation and characterization of SLNs and nanovectors

SLNs were synthesized with DOTAP, Tween 80 and Precirol® ATO5 by a hot-melt emulsification technique [4]. To form the nanovectors, a pDNA encoding α-Gal A was mixed with protamine, a polysaccharide (dextran – DX or galactomannan – GM) and SLNs. Formulations were characterized in terms of size, polydispersity index and zeta potential.

2.2. In vitro transfection studies in Hep G2

To evaluate the transfection efficacy Hep G2 cells were treated with the nanovectors and α-Gal A activity was quantified in the culture medium by a fluorimetric assay, 3 and 5 days after the addition of the formulations. Lipofectamine® 2000 Transfection Reagent was used as positive control of transfection.

3. Results and discussion

The hydrodynamic diameter of the SLNs was 93 nm and the surface charge +73 mV. The nanovectors prepared with the two different polysaccharides presented a size in the nanometre range suitable for intravenous administration; 104 nm the formulation with DX and 107 nm the one with GM. Both nanovectors had a positive zeta potential (+43 and +31 mV,

respectively), which favours the interaction with the negatively charged cell membrane. The polydispersity index of all the formulations was below 0.3.

Both nanovectors were able to increase significantly enzyme activity over untreated cells 3 and 5 days after the treatment (Figure 1). Nevertheless, the nanovector formulated with GM resulted more effective than that with DX, increasing enzyme activity 685-fold with respect to non-treated cells at day 5. Moreover, 5 days after the treatment the vector with GM incremented the enzyme activity 1.5 times over the positive control Lipofectamine® 2000. The high efficacy of the GM nanovector is probably due to the presence of galactose and mannose groups of the GM that bind and drive the vector through liver-specific receptors [5].

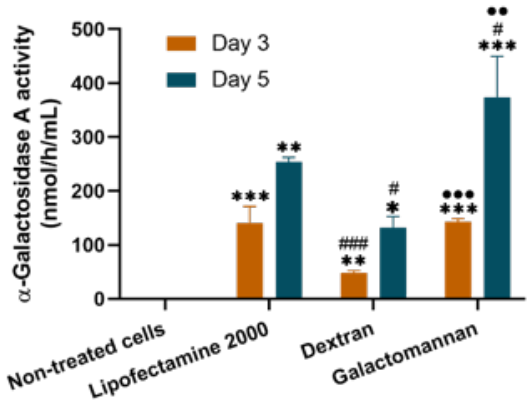

Fig. 1. α-Galactosidase A activity in the supernatant of Hep G2 cells 3 and 5 days post-transfection. Data represent mean ± standard deviation of three biological replicates. Enzyme activity of non-treated cells < 1 nmol/h/mL. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ with respect to non-treated cells. # $p < 0.05$, ### $p < 0.001$ with respect to Lipofectamine® 2000 at the same sampling time. •• $p < 0.01$, ••• $p < 0.001$ with respect to dextran nanovector at the same sampling time.

4. Conclusions

Herein, we have developed and evaluated SLNs-based nanovectors decorated with two different polysaccharides able to increase α-Gal A activity in hepatocytes. The nanovectors formulated with DX and GM presented suitable physicochemical characteristics to reach hepatocytes in vivo after intravenous administration. Both formulations were able to increase α-Gal A activity in the supernatant of treated hepatocytes. However, the nanovector containing GM on the surface was more effective, suggesting a key role of this polysaccharide in non-viral liver-targeted vectors for the treatment of FD.

Acknowledgements

RODRÍGUEZ-CASTEJÓN, Julen thanks the Basque Government (PRE_2020_2_0120) and GÓMEZ-AGUADO, Itziar and BERAZA-MILLOR, Marina thank the University of the Basque Country UPV/EHU (PIF17/067 and PIFG19/36, respectively) for their research grants. This research has been funded by MCIU/AEI/FEDER, UE (RTI2018-098672-B-I00) and by the University of the Basque Country UPV/EHU (GIU20/048).

References

1. Rodríguez-Castejón J, Alarcia-Lacalle A, Gómez-Aguado I, Vicente-Pascual M, Solinís Aspiazu MA, del Pozo-Rodríguez A, Rodríguez-Gascón A. α-Galactosidase A augmentation by non-viral gene therapy: evaluation in fabry disease mice. Pharmaceutics [Internet]. 2021 May 21 [cited 2021 Sept 16];13(6):771. Available from: https://doi.org/10.3390/pharmaceutics13060771

2. Aravalli RN, Belcher JD, Steer CJ. Liver-targeted gene therapy: approaches and challenges. Liver Transpl [Internet]. 2015 March 30 [cited 2021 Sept 16];21(6):718-737. Available from: https://doi.org/10.1002/lt.24122

3. Tapeinos C, Battaglini M, Ciofani G. Advances in the design of solid lipid nanoparticles and nanostructured lipid carriers for targeting brain diseases. J Control Release [Internet]. 2017 Oct 28 [cited 2021 Sept 16];264:306-332. Available from: https://doi.org/10.1016/j.jconrel.2017.08.033

4. Gómez-Aguado I, Rodríguez-Castejón J, Beraza-Millor M, Vicente-Pascual M, Rodríguez-Gascón A, Garelli S, Battaglia L, del Pozo-Rodríguez A, Solinís MA. mRNA-based nanomedicinal products to address corneal inflammation by interleukin-10 supplementation. Pharmaceutics [Internet]. 2021 Sept 15 [cited 2021 Sept 16];13(9),1472. Available from: https://doi.org/10.3390/pharmaceutics13091472

5. Chen F, Huang G, Huang H. Sugar ligand-mediated drug delivery. Future Med Chem [Internet]. 2019 Nov 13 [cited 2021 Sept 17];12(2),161-171. Available from: https://doi.org/10.4155/fmc-2019-0114

Este trabajo debe ser citado como:

Rodríguez Castejón J, Gómez Aguado I, Beraza Millor M, Solinís MA, Del Pozo Rodríguez A., Rodríguez Gascón A. Functionalised SLNs-based nanovectors for gene therapy in Fabry disease: The liver as an α-Galactosidase A factory. Rev Esp Cien Farm. 2021;2(2):46-8.