rescifar REVISTA ESPAÑOLA DE CIENCIAS FARMACÉUTICAS

Substrate reduction therapy based on siRNA for Fabry Disease

Beraza Millor Marina *[1,2], Miranda Jonatan[3,4], Rodríguez Castejón Julen[2], Gómez Aguado Itziar[1,2], del Pozo Rodríguez Ana[1,2], Rodríguez Gascón Alicia[1,2], Solinís María Ángeles[1,2]

[1] Pharmacokinetics, Nanotechnology and Gene Therapy (PharmaNanoGene), Faculty of Pharmacy, Centro de Investigación Lascaray Ikergunea, University of Basque Country, UPV/EHU, Vitoria-Gasteiz.
[2] Bioaraba, Microbiology, Infectious Disease, Antimicrobial Agents and Gene Therapy, Vitoria-Gasteiz, Spain.
[3] GLUTEN3S Research Group, Faculty of Pharmacy, University of Basque Country, UPV/EHU, Vitoria-Gasteiz.
[4] Bioaraba, Nutrition and Food Safety, Vitoria-Gasteiz, Spain.
*Correspondencia: marina.beraza@ehu.eus

1. Introduction

Fabry Disease (FD) is a lysosomal storage disorder caused by mutations in GLA gene, resulting in an insufficient α-galactosidase A (α-Gal A) enzyme activity. Consequently, there is a progressive accumulation of glycosphingolipids, particularly globotriaosylceramide (Gb3) and its derivated lyso-Gb3 in vascular endothelial and smooth-muscle cells, specially in human heart and kidney [1].

The available therapeutic options are limited to enzyme replacement therapy (Replagal® and Fabrazyme®), and the oral chaperone Migalastat®. These therapies increase α-Gal A activity, reducing the levels of Gb3 and lyso-Gb3. In this sense, substrate reduction therapy (SRT) is a new approach for limiting the formation of glycosphingolipids. SRT targeted to Gb3 synthase (encoded by the A4GALT gene) has been proposed as a new strategy for FD, as it leads to a reduction in Gb3 avoiding the depletion of other glycosphingolipids which do not accumulate in FD [2].

Silencing therapies based on small interfering RNA (siRNA) are promising options as SRT tools. siRNA is a double-stranded RNA molecule, 21-25 nucleotides in length, able to cleave its complementary mRNA. The use of siRNAs requires the development of a vector able to protect it and to facilitate its interaction with the target cell, affording an adequate intracellular disposition of the nucleic acid. Lipid nanoparticles (LNP) are among the most widely non-viral nucleic acids carriers. LNP show several advantages, such as low or absence of in vivo toxicity, good long-term stability and the possibility to autoclave and sterilize. In fact, in 2018 FDA and EMA approved the first siRNA product, Onpattro®, and it was formulated in LNP.

In this work, we have evaluated four different siRNA molecules targeted to the mRNA expressed by A4GALT gene. siRNAs were formulated in LNP as well as in a commercial transfection reagent. The silencing capacity was evaluated in IMFE-1 cells, a cellular line derived from primary endothelial cells obtained from FD patients.

2. Materials and methods

2.1. Materials

Four siRNAs targeted to A4GALT gene differing in the nucleotide composition, and the DharmaFECT® Transfection Reagent were provided by Horizon Discovery (Waterbeach, England). DOTAP was obtained from Avanti Polar-lipids, Inc (Alabaster, AL, USA). Gattefossé

(Madrid, Spain) kindly provided Precirol® ATO 5 (Madrid, Spain). Real Time RT-PCR products were all obtained from Roche Diagnostics (Mannheim, Germany). All other reagents were analytical grade.

2.2. Methods

2.2.1. Preparation of the formulations

LNPs were prepared using two different techniques: solvent evaporation/emulsification and hot-melt emulsification [3]. The final vectors were decorated with dextran and protamine. Formulations were characterized in terms of size, polydispersity index and zeta potential. DharmaFECT® Transfection Reagent was formulated as indicated by its own protocol.

2.2.2. Silencing studies and viability analysis

IMFE-1 cells were transfected with four different siRNAs formulated in LPN as well as in the commercial transfection reagent. Forty-eight hours after the addition of the formulations silencing efficacy was measured by real time RT-PCR. Briefly, total RNA was extracted from the cells using a High Pure RNA Isolation Kit. RNA was then reverse-transcribed by a First Strand cDNA Synthesis Kit. cDNA was amplified and quantified using the LightCycler® FastStart DNA Master SBR Green I, using the specific primer sets to quantify A4GALT gene and β-actin as the endogenous reference. The analysis was performed by the LightCycler® 2.0 system (Roche). For viability analysis MTT was performed 24 hours after transfection.

3. Results and discussion

LNP vectors prepared by solvent evaporation/ emulsification showed a particle size of 300 nm and a positive superficial charge of +36 mV. Vectors based on LNP prepared by hot-melt emulsification showed a lower size and zeta potential, 140 nm and +12 mV, respectively. Due to instability problems, vectors containing the LNPs prepared by solvent evaporation/ emulsification were discarded.

Different concentrations of siRNA (15-50 nM) were tested in IMFE-1 cells in order to evaluate the effect of the dose on the efficacy. Silencing studies showed that all except one (siRNA 4) were able to inhibit A4GALT gene. siRNA 1 was able to silence the A4GALT gene but in a lower extent than siRNA 2 and 3. When siRNA 2 and 3 were formulated in LNP, they showed their maximum inhibitory capacity (80 %) at a concentration of 25 nM (Figure 1). However, when siRNA 3 is formulated with the commercial reagent, 50 nM concentration was necessary for a high inhibition.

Cell viability was higher than 90 % when cells were treated with the siRNA containing LNP regardless siRNA concentration. However, with the commercial reagent cell viability decreased to around 60 % when a 50 nM concentration was assayed.

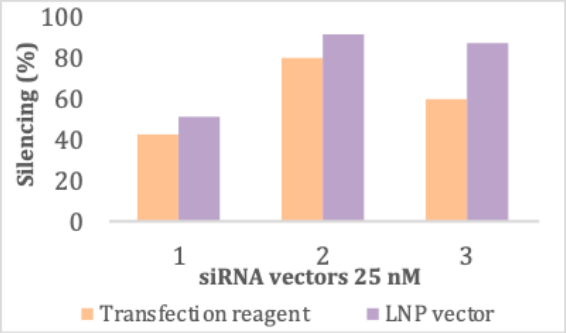

Figure 1. Gene silencing efficacy of different siRNAS.

4. Conclusion

siRNA-vectors containing LNP prepared by hot-melt emulsification present a lower particle size and a better stability compared to those prepared by solvent evaporation/emulsification.

LNP-based vectors provided a high gene silencing efficacy without affecting IMFE-1 cell viability.

Substrate reduction therapy based on the administration of siRNA targeted to Gb3 synthase enzyme formulated in LNP could be a feasible strategy to tackle FD.

Acknowledgements

The author wants to thank the UPV/EHU for her research grant (PIFG19/36). This research has been funded by MCIU/AEI/FEDER, UE (RTI2018-098672-B-I00) and by the UPV/EHU (GIU20/048).

References

1. Rodriguez-Castejón et al. α-Galactosidase A augmentation by non-viral gene therapy: evaluation in Fabry Disease mice. Pharmaceutics. 2021:13(6):771.

2. Miller JJ et al. Progress in the understanding and treatment of Fabry disease. Biochim Biophys Acta Gen Subj. 2020:1864(1):129437.

3. Gómez-Aguado I et al. mRNA-based nanomedicinal products to address corneal inflammation by interleukin-10 supplementation. Pharmaceutics. 2021;13(9):1472.

Este trabajo debe ser citado como:

Beraza Millor M, Miranda J, Rodríguez Castejón J, Gómez Aguado I, Del Pozo Rodríguez A, Rodríguez Gascón A, Solinís MA. Substrate reduction therapy based on siRNA for Fabry Disease. Rev Esp Cien Farm. 2021;2(2):84-6.