Improvement of the Enzymatic Activity of α-Galactosidase Using Nanovesicles with application to Fabry Disease treatment

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Fabry disease is a rare inherited disease caused by loss of function of the enzyme α-Galactosidase A (GLA) [1]. Commercially available treatments are based on the intravenous administration of GLA demonstrating positive short-term effect, reducing the progression of the disease and improving the quality of life in patients. However, GLA replacement therapy exhibits drawbacks such as the degradation of the exogenously administered enzyme, its limited efficacy in patients with an advance stage of the disease and the extremely high cost of the treatment. In order to improve the delivery efficacy and the systemic circulation of the current treatment, nanoliposomes containing GLA were prepared as novel drug delivery systems (DDS). The incorporation of GLA in liposomes was obtained following the DELOS-SUSP process based on the use of compressed CO_2 [2] as co-solvent (**Figure 1**). Liposomes were constituted from phospholipids (DPPC) and cholesterol-based compounds. In addition, c(RGDfK) peptide ligand was incorporated in the membrane bilayer of the vesicles to enhance the targeting and the uptake efficiency of the GLA-loaded conjugates to the diseased cells. The conjugate was further characterized to obtain information on its physico-chemical characteristics and morphology entrapment efficiency and also biological efficacy and cell uptake. Through the DELOS-SUSP process, nanometric liposomes containing GLA were successfully prepared with an entrapment efficiency of about 40 %. *In vitro* efficacy studies in GLA deficient cells of Fabry KO mice showed that the GLA-nanoformulations were able to reduce lysosomal Gb3 deposits more efficiently than the free enzyme in agreement with a greater specific activity also encountered (**Figure 2**). This finding indicates that (i) such multifunctional nanovesicles are uptake by GLA deficient cells, (ii) the GLA-nanovesicles reach the lysosomal compartment, and (iii) the cargo (GLA) is efficiently released so that the GLA activity in the cells is restored. The results obtained prove the great potential of DELOS-SUSP method for the production of new nanomedicine candidates based on enzyme-nanovesicle conjugates. The development of these new GLA-nanoconjugates up to the end of the regulatory preclinical phase will be carried out under the frame of the European Smart-4-Fabry project (H2020-NMBP-2016-2017 GA 720942).

References

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[3] Cabrera, I.; *et al. Adv. Healthcare Mater.* 7 (2016) 829-40.

Figures

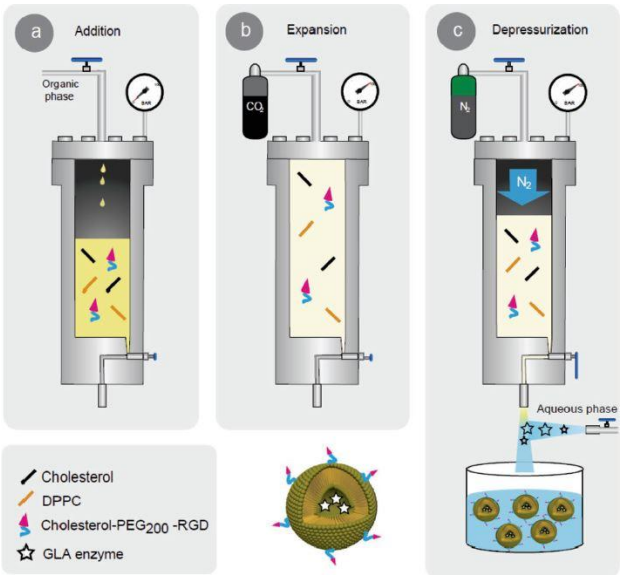

Figure 1. Nano-GLA multifunctional nanoformulation, manufactured by the DELOS-SUSP platform.

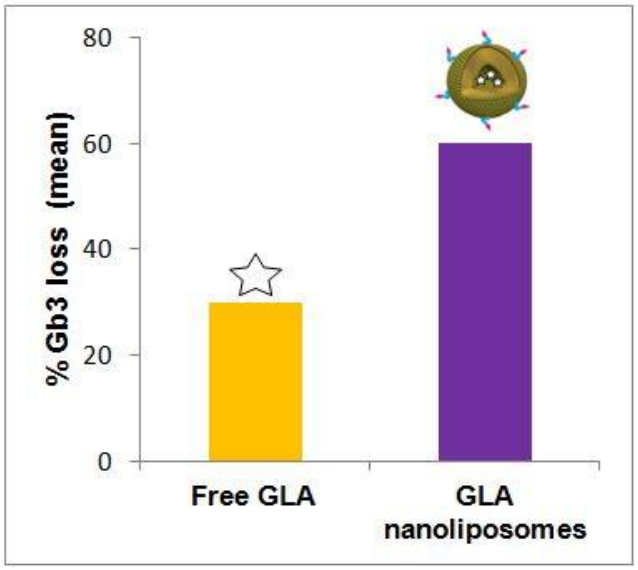

Figure 2. Effect of free GLA and GLA-Nanoliposomes in the reduction of Gb3 deposits in aortic endothelial cells of Fabry KO mice (right). Represented values correspond to mean.