P3.2: Targeted cathelicidin nanomedicines as novel glucoregulator for diabetes therapy

C. Cristelo[1;2]*, F. M. Gama[2], B. Sarmento[1]

[1] *i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal;*
[2] Centro de Engenharia Biológica, Universidade do Minho, Braga, Portugal
*e-mail: cecilia.cristelo@i3s.up.pt

Introduction

Type 1 diabetes (T1D) is an autoimmune condition resulting in the destruction of the insulin-producing β cells. With no cure available for this disease, patients depend on insulin administration to control glycaemia. The recovery of β cells by promoting its replication, neogenesis or by reprogramming of other pancreatic cells, offers a promising strategy to revert T1D. Cathelicidin is an antimicrobial peptide that has been shown to improve β cell function and neogenesis. This work aims to develop a delivery system for a cathelicidin-derived peptide specifically to β cells, aiming at promoting their function and increase cellular mass. To achieve the specific delivery to β cells, the peptide was loaded on PLGA nanoparticles (NPs). The NPs were surface-decorated with exenatide, an agonist of the GLP-1 receptor, expressed by β cells.

Materials and Methods

NPs were produced by double emulsion solvent evaporation and characterized using dynamic light scattering (DLS), nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). The association efficiency and drug loading of the cathelicidin-derived peptide (LLKKK18) were determined using fluorescamine assay. The effects of the nanoformulation on glucose-stimulated insulin secretion (GSIS) by INS-1E cells were evaluated by ELISA, and on cell viability using MTT. The biological interaction was studied using flow cytometry and fluorescence microscopy.

Results and Discussion

NPs loaded with the cathelicidin-derived peptide LLKKK18 and surface-functionalized with exenatide presented a mean size around 100 nm, a narrow size distribution (PdI of 0.14 ± 0.02), zeta potential of -9.6 ± 0.7mV and stability up to 20 days at 4ºC in PBS. The association efficiency and LLKKK18 loading were 85% and 4%, respectively. LLKKK18 was released very slowly from PLGA NPs in PBS. In the first 24h around 20% LLKKK18 was released, and it nearly stabilized in the first 5 days, after which it started to slowly increase.

Soluble LLKKK18 improved GSIS; however, when loaded in NPs it had no significant effect on insulin release. NP functionalization with exenatide showed a slight increase of insulin release compared to non-funcionalized NPs under high glucose concentration. Soluble LLKKK18 significantly increased the cellular viability after 96h of incubation with INS-1E cells; the study of the effect from LLKKK18 NPs on cell viability is ongoing.

Different strategies have been undertaken to improve β cell targeting. NPs were functionalized with exenatide with or without a PEG spacer before C-terminal cysteine, for site-specific conjugation to maleimide. Functionalization was performed either post NP production or using a previously functionalized polymer. In all strategies, NP interaction with INS-1E cells was minor and functionalization did not favour interaction. The expression of GLP-1R by INS-1E cells was slightly greater than the negative control (L929 cells), but still underexpressed. Ongoing studies are being carried out using a GLP-1R transfected cell line.

Conclusions

We successfully obtained cathelicidin-loaded, and exenatide-functionalized NPs with a monodisperse distribution and a slow release. The functionalization of NPs was not efficient in promoting the interaction with β cells possibly related with the low expression of the GLP-1R by INS-1E cells. The ability of the nanoformulation to to improve GSIS and replication is still to be clarified.

Acknowledgments

This work was supported by Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (POCI-01-0145-FEDER-007274). Cecília Cristelo gratefully acknowledges FCT for financial support (fellowship SFRH/BD/139402/2018).