Dissertation Project – 2nd Cycle

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TITLE: Role of Islet Amyloid Polipeptide in the progression of Diabetes Mellitus and identification of compounds preventing its aggregation

BACKGROUND

Islet Amyloid PoliPeptide (IAPP) is a hormone co-secreted with insulin by the pancreatic islets of Langerhans β-cells upon glucose stimulation. It participates in normal glucose regulation, inhibiting insulin and glucagon secretion by the islets. Monomeric IAPP forms intermediate structures leading to the formation of amyloid fibres, which deposit in the surrounding tissues. These amorphous structures are histopathological hallmarks of Type 2 Diabetes Mellitus (T2DM). Though the ultimate cause of T2DM remains uncertain, it is recognized that IAPP misfolding and aggregation is critical for β-cells demise leading to a scenario of impaired insulin secretion and disease progression. The discovery of elevated IAPP levels, increasing the risk of aggregation, in a sub-population of young newly diagnosed Type 1 Diabetes Mellitus (T1DM) patients has put forward IAPP toxicity also in the list of putative pathological factors of T1DM. Additionally, IAPP amyloid is observed in conditions associated to β-cell stress such as whole pancreas and islet transplantation, reducing insulin secretion and compromising the efficacy of these interventions. Thus, the study of the molecular mechanisms underlying IAPP proteotoxicity represents a novel window of research in DM impacting various aspects of the disease.
Compounds modulating pathways associated to IAPP pathogenesis, i.e. oxidative stress, inflammatory processes and proteostasis, have potential therapeutic application for DM. Phenolic compounds, given their pleiotropic effects, fit as potential molecules to be developed for DM. Indeed, resveratrol, myricetin and oleuropein aglycone are described to interfere with IAPP amyloid fibril formation rescuing cells from the deleterious effects of these species. Preliminary analysis obtained in the Molecular Nutrition & Health Lab revealed that phenolic metabolites, detected in human plasma after ingestion of a polyphenol-rich puree, attenuate inflammation in rat primary cells and modulate expression of the glucose transporter GLUT1, revealing their potential as DM drugs or leads for therapeutic molecules.

OBJECTIVES

DM therapeutics focus on the maintenance of euglycemia, with little efforts made to develop strategies to prevent pancreatic β-cells death. The increase of IAPP levels and its aggregation have been increasingly regarded as critical pathological processes associated to β-cell death in T2DM, T1DM and in islet-transplanted individuals. This project focuses on the exploitation of versatile IAPP-aggregation models (yeast and beta-pancreatic cell lines) to provide beyond the state-of-art knowledge of IAPP-driven DM mechanisms to be translated into novel therapeutic strategies.
The main objectives of the proposal are:

1. Providing pre-clinical support for the pathological role of IAPP aggregation in the early progression of T1DM and T2DM in rodent models of both diseases;
2. Identification phenolic metabolites affecting IAPP aggregation in humanized IAPP yeast models
3. Validating the major findings in β-pancreatic cell lines

PROJECT DESCRIPTION

The present project was distinguished by "Sociedade Portuguesa de Diabetologia – SPD" and will involve collaborative work with the consortium partners.

TASK 1: IAPP AGGREGATION AS A RISK FACTOR FOR DM

The goal of this task is to provide clinical support for the increase of IAPP levels as a relevant pathological process in T1DM and T2DM.

STRATEGY: Even though rodent IAPP does not aggregate, evaluation of IAPP plasma levels in T1DM rodent models will provide valuable information regarding IAPP levels in the early events of T1DM. IAPP levels will be monitored (by ELISA) in the plasma of recent onset T1DM (Ins2 Akita mouse model), T2DM (ZDF rat model) and healthy control animals. Parameters such as plasma concentration of glucose and inflammatory markers such as NO and TNFα will be also evaluated (standard biochemical assays or ELISA) to allow correlation studies.

TASK 2: PHENOLIC METABOLITES AS MODIFIERS OF IAPP TOXICITY

Among phenolic metabolites previously identified in our team, two revealed potential activities for DM. Catechol-O-sulfate inhibited expression of GLUT1 in human epithelial cells subjected to hyperglycaemia and Pyrogallol-O-sulfate was shown to be a potent attenuator LPS-induced inflammation in rat cell line and Wistar primary cells as revealed by decreased NFκB nuclear accumulation and 50% reduction of TNFα levels (Garcia et al., unp data).

STRATEGY: Yeast models recapitulating human IAPP (hIAPP) proteotoxicity (recently developed in our laboratory) will be used to screen for protective phenolic compounds by means of growth assays. Investigation of the molecular mechanisms underlying phenolics-driven protection will be carried out using qRT-PCR, flow cytometry, immunoblot analysis and fluorescence microscopy) to monitor hIAPP expression, protein levels and dynamics.

TASK 3: VALIDATION IN MAMMALIAN CELL MODELS

The major findings obtained in Task 2 will be validated in β-pancreatic cell lines, which represent a superior and more physiological model of DM.

STRATEGY: β-pancreatic cell lines overexpressing hIAPP will be used to validate the protective activity of the most promising phenolic compounds identified in Task 2. Cell viability will be monitored using the CellTiter Assay and state-of-art cellular and molecular biology approaches, including confocal fluorescence microscopy, will be used to monitor cell physiology and protein dynamics.

TIMELINE (use fill tool for the cells)

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1										
Task 2										
Task 3										
Thesis										