Abstract

The 37-amino acid residue peptide human amylin, also known as human islet amyloid polypeptide (hIAPP), forms amyloid protein deposits in the pancreas which are regarded to play a key role in the pathogenesis of type two diabetes mellitus. The factors promoting hIAPP aggregation are still not completely clear, however rising evidence indicates that oligomeric aggregates are cytotoxic to β-cells, probably by interaction with and subsequent disruption of β-cell membranes. Consequently, increasing research is aimed at developing inhibitors of cytotoxic amyloid aggregation of hIAPP. In the last times great attention has been paid to the multiple health benefits coming from a diet rich in antioxidants. Cytotoxicity of amyloid aggregates is frequently associated to early modifications of the intracellular redox *status*, and this has raised interest on the possible protection provided by natural antioxidants. In this respect, extra virgin olive oil is attracting great attention as an important source of polyphenols.

In this study we have shown that oligomers of hIAPP are highly toxic to cultured mammalian cells and that oleuropein aglycon, the main phenolic component found in Mediterrean extra virgin olive oil, can reduce the cytotoxicity of human amylin.

We tested the cytotoxicity of hIAPP aggregates obtained in the presence and in the absence of oleuropein aglycon on RIN-5F rat pancreatic β-cells by the MTT reduction inhibition assay. Human amylin treatment resulted in a highly significant impairment of cells viability with respect to controls, while the viability of cells treated with hIAPP incubated with oleuropein aglycon, was not significantly different from that of control cells. Then we have shown by immunofluorescence analysis that this protective action may come from the ability of oleuropein aglycon to inhibit the interaction between aggregates and the cell surface, and this had led us to hypothesise that hIAPP aggregates grown in the absence or in the presence of oleuropein are somehow structurally different.

This interaction leads to membrane destabilization with consequent membrane permeabilization, oxidative stress, and eventually cell death by apoptosis. In fact we have verified the apoptotic response, in terms of caspase-3 activation, in cells treated with hIAPP aggregates, while there was no increase of caspase-3 activity in cells treated with hIAPP aggregates grown with oleuropein. These results confirm the protective action of oleuropein aglycon showed by the MTT data and the necessity of aggregates-membrane interaction to induce toxicity.

To further confirm the latter hypothesis, we have carried out an *in vitro* experiment, incubating synthetic phospholipid unilamellar vesicles (DOPS:DOPC = 3:7) with hIAPP aggregates aged in the presence or in the absence of oleuropein aglycon. We have shown that the toxic hIAPP assemblies induce a significant calcein release from negatively charged phosphatidylserine vesicles, whereas no significant permeabilization was induced by the aggregates grown in the presence of oleuropein .

Finally, structural studies performed by ThT assay, Circular Dichroism and Electron microscopy have indicated that oleuropein aglycon interferes with the first phases of hIAPP aggregation, during which cytotoxic hIAPP aggregates are formed, resulting in a different path of amyloid aggregation.

Overall, our results suggest both a possible beneficial effect coming from extra virgin olive oil consumption in the prevention of type two diabetes and a pharmacological use of oleuropein in the treatment of this disease.