Maintaining INS-1 cell function and enhancing cell survival by inhibiting IAPP fibril formation with novel Compound-A

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Introduction and Background

Islet Amyloid Polypeptide (IAPP, amylin)

IAPP is normal beta-cell hormone co-secreted and co-stored with Insulin in the secretory vesicles. IAPP helps in gastric emptying, glucose homeostasis, and suppression of glucagon release.

Human IAPP (hIAPP) accumulation

IAPP has amyloidogenic sequence and forms amyloid plaques in the Islets of Langerhans in diabetes patients. hIAPP forms into unbranched fibrils of an indefinite length and a diameter of 7-10 nm. The built-in monomers are assembled into sheet structure arranged perpendicularly to fibril axis. Amyloid fibril formation is a self-driven process. After initiation, fibril formation continues as long as the precursor is present in sufficient quantities.

Hypothesis and Objective

Hypothesis

This study hypothesised that fibrillar hIAPP contributes profoundly to INS-1 cell death, and by blocking the formation of oligomeric fibrillar hIAPP protein will decrease the cytotoxic effects of hIAPP, thereby enhancing INS-1 cell survival.

Objective

The objective of the study was to see notable differences between the use of Compound-A on INS-1 cells function and proliferation after seven days of incubation with hIAPP as a proof of principle.

Experimental Methodology

• INS-1 cells were cultured with exogenous hIAPP either in the presence or absence of Compound A (10μM) for 7 days.
• The cultured cells were categorized as models for three different conditions: (i) Control (+hIAPP, -Compound-A); (ii) Non-treatment (+hIAPP, -Compound-A); (iii) Treatment (+hIAPP, +Compound-A).
• Slides of fixed INS-1 cells from cell culture were double immunostained for Insulin/TUNEL (apoptosis) and Insulin/PCNA (proliferation).
• The proportion of TUNEL positive cells and PCNA positive cells were quantified in INS-1 cell micrographs
• One-way ANOVA and student t-test were the statistical tests done. P-value less than 0.05 (p < 0.05) was deemed statistically significant.

Results

INS-1 cells exposed to hIAPP incubated with Compound-A significantly reduced cell apoptosis after 7 days

Figure 2. Representative micrographs from three conditions: a) Day 0: Control; b) Day 7: Non-treatment; c) Day 7: Treatment. Green = Insulin Red = TUNEL (apoptosis)

Summary and Conclusion

After seven days of culture in the presence of hIAPP, cells with Compound-A were significantly enhanced the function of INS-1 cell survival.

Future and ongoing work

Future studies will focus working with Compound-A on pancreatic beta cells, mechanism of action of Compound-A, structure of Compound-A and pharmacological aspects of Compound-A.

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References:

