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.

hIAPP fibril aggregation and pancreatic beta-cell death
hIAPP contributes to progressive beta-cell dysfunction and death in diabetic patients. There is strong evidence that hIAPP aggregation exacerbates pancreatic dysfunction via membrane-disrupting oligomers. Endoplasmic reticulum (ER) initiates unresolvable unfolded protein response (UPR) leading to apoptosis. Consequently, pancreatic beta cell mass and function decreases over time.

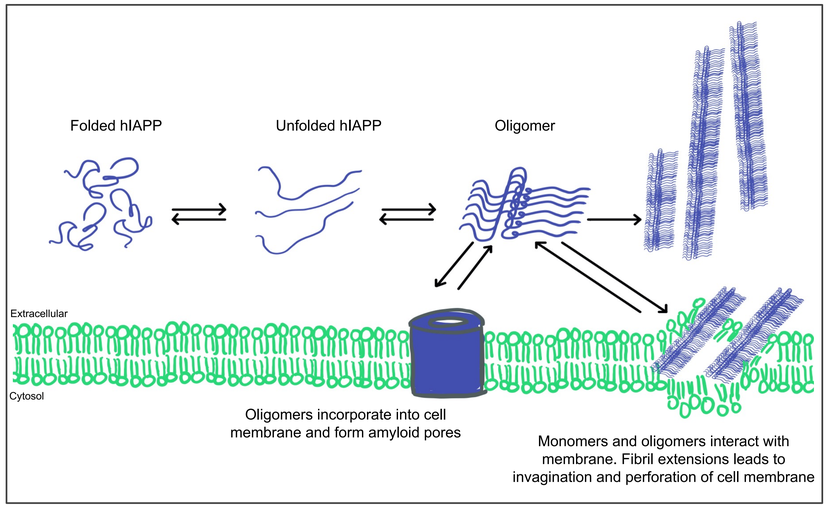

Figure 1. Schematic of hIAPP fibril formation into toxic oligomeric forms.

Compound-A
Compound-A is a naturally occurring, novel compound which inhibits IAPP fibril formation exogenously. The chemical structure and mechanism of action of Compound-A is being studied. The identity and source of Compound-A is kept confidential because the studies are underway at preliminary stages.

References:

Kiriyama, Y., and Nochi, H. (2018). Role and cytotoxicity of amylin and protection of pancreatic islet beta-cells from amylin cytotoxicity. *Cell* 7:95.

Pithadia, A., Brender, J. R., Fierke, C. A., and Ramamoorthy, A. (2016). Inhibition of IAPP aggregation and toxicity by natural products and derivatives. *J. Diabetes Res.* 2016:2046327.

Ridler, C. Misfolded diabetes-mellitus peptide seeds amyloid-β aggregation. *Nat Rev Neurol* 13, 128 (2017).

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 fixated 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

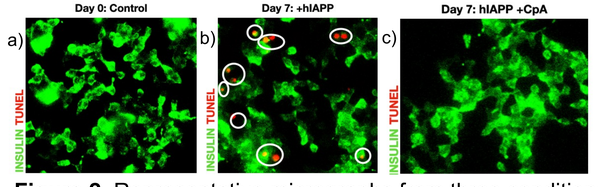

Figure 2. Representative micrographs from three conditions:
a) Day 0: Control; **b)** Day 7: Non-treatment; **c)** Day 7: Treatment.
Green = Insulin Red = TUNEL (apoptosis)

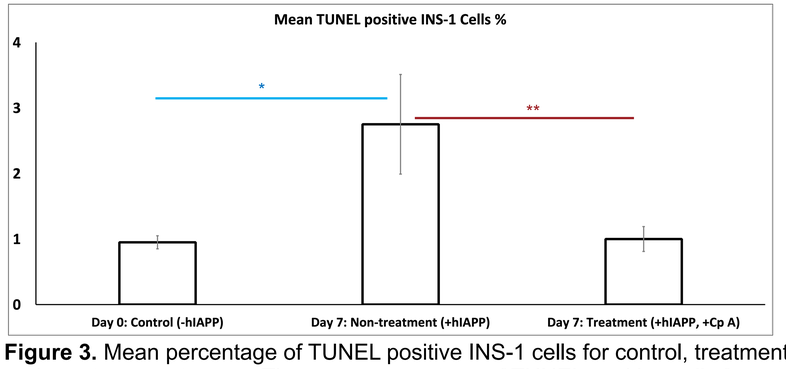

Figure 3. Mean percentage of TUNEL positive INS-1 cells for control, treatment and non-treatment group. The mean percentage of TUNEL positive cells for control, non-treatment and treatment group were 0.95% ± 0.1%, 2.75% ± 0.76%, and 1% ± 0.2%, respectively. (t-test, * = $p < 0.001$, ** = $p < 0.01$)

Results (Continued)

Compound-A significantly enhanced the function of INS-1 cells after 7 days of cell culture in the presence of hIAPP

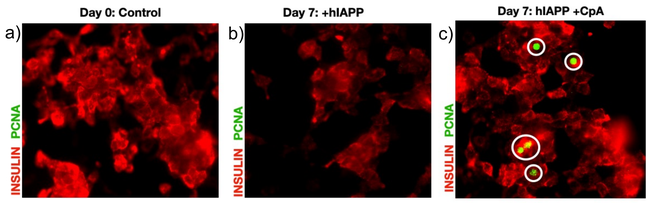

Figure 4. Representative micrographs from three conditions:
a) Day 0: Control; **b)** Day 7: Non-treatment; **c)** Day 7: Treatment.
Red = Insulin Green = PCNA (proliferation)

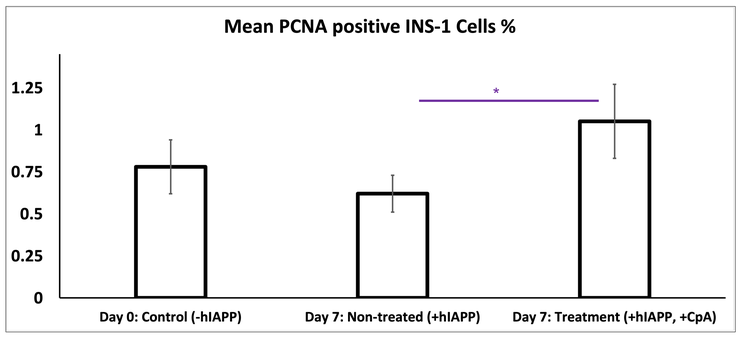

Figure 5. Mean percentage of PCNA positive INS-1 cells for control, treatment and non-treatment group. The mean percentage of PCNA positive cells for control, non-treatment and treatment group were 0.78% ± 0.16%, 0.62% ± 0.1%, and 1% ± 0.15%, respectively. (t-test, * = $p < 0.05$)

Summary and Conclusion

After seven days of culture in the presence of hIAPP, cells with Compound-A had:
- Significant reduction in TUNEL positive INS-1 cells suggesting that the INS-1 cell survival was enhanced.
- Significant hike in PCNA positive INS-1 cells suggesting that the INS-1 cell function was preserved.

Hence, this study established the proof of principle that blocking hIAPP fibril formation by Compound-A can serve as possible therapeutic agent.

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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