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Zn(II), Cu(II), Sn(II), and Ni(II) and other metal cations do not prevent the aggregation of hIAPP

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Zn(II), Cu(II), Sn(II), and Ni(II) and other metal cations do not prevent the aggregation of hIAPP

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Introduction

It is estimated that currently 8.3% of the U.S. population has diabetes, with nearly 2 million new cases each year (American Diabetes Association, 2013). The total cost of treating diagnosed diabetes is $245 billion annually (American Diabetes Association 2013), with no cure known. As type II Diabetes progresses, B-cell function decreases and apoptosis increases (Butler *et al*., 2003). Human Islet Amyloid Polypeptide (hIAPP) has been found to form plaques in the islets of type II Diabetes patients (Kahn *et al*., 1999), and hIAPP has been shown to be toxic to the B-cells, causing apoptosis (Lorenzo *et al*., 1994).

IAPP is a native protein in the body that regulates blood sugar by slowing gastric emptying and promoting satiety (Westermark *et al*., 2011). As B-cells die, the remaining islets cannot keep up with insulin release, causing or contributing to hyperglycemia. Zinc has been shown to slow the aggregation of hIAPP likely due to an interaction with His18 of the protein (Brender *et al*., 2010). Cu(II) (Ward *et al*., 2007, Brender *et al*., 2010) and gold complexes (He *et al*., 2015) have also been demonstrated to potentially inhibit the aggregation of hIAPP *in vitro*. However, complete studies should look at atomic force microscopy (AFM) pictures to exclude the possibility of interference of dye-fibril binding (Hudson *et al*., 2009).

This study set out to test the potential for inhibition of hIAPP aggregation by incubation with metal cations. Fluorimetry using Thioflavin T (ThT), which binds to pleated B-sheets in amyloid fibrils (Groenning *et al*., 2007), was used as a preliminary test. AFM, which scans slides to show fibril structure, was used to confirm positive results for ThT.

Materials and Methods

Preparation of hIAPP solutions

Human hIAPP (hIAPP) was ordered from _____ (batch no.) and _____ (batch no.) suspended in hexafluoroisopropanol and frozen at -80 C until each run. At the beginningof each ThT assay, 375 uL (1st batch) or 325 uL (2nd batch) of hIAPP was added to glass culture tubes and the solvent evaporated off in a Speed-Vac (Thermofischer Scientific DNA120) before metal solutions were added.

Preparation of metal solutions

The appropriate amount of metal compound to achieve a concentration of 10 mM was added in a solution of 20 mM TRIS (pH=7.4).

Thioflavin T assays

15 uL metal solution was added to the hIAPP in culture tubes with 135 uL TRIS buffer; 150 uL TRIS buffer was used each time as a control. The samples were incubated in a shaker (New Brunswick Scientific Innova 4000) and samples were removed to test ThT fluorescence. 17 uL metal-IAPP solution was added with 663 uL of 75 uM ThT to fluorescence cuvettes; the fluorescence was measured in a fluorescence spectrophotometer (Hitachi F-7000) with an excitation wavelength of 450 nm; ThT fluorescence was measured at 488 nm. A t-test was used to determine whether metals significantly lowered ThT fluorescence.

Atomic force microscopy

Metals that successfully reduced ThT fluorescence compared to their respective controls were run on an atomic force microscope to confirm the absence of fibrils.

Results

Thioflavin T Assays

Cu(II) and Ni(II) were the only two metals to have significantly lower Thioflavin T concentrations (t-test: p=0.000278, dof=3; p=0.049, dof=3, respectively; figs. 1,2).

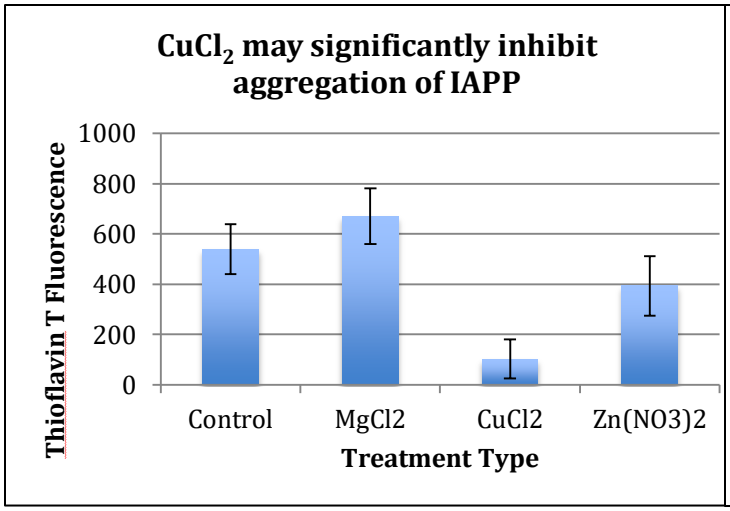

Figure 1: Cu(II) significantly inhibits ThT fluorescence; Zn(II) may inhibit; Mg(II) does not.

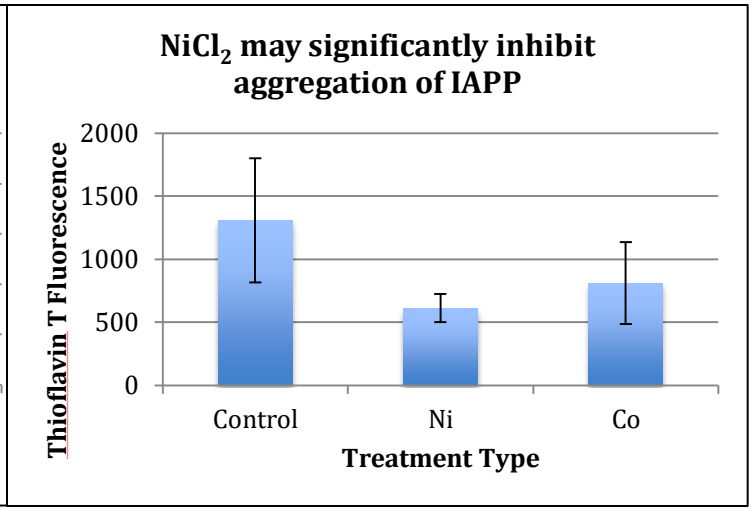

Figure 2: Ni(II) significantly inhibits ThT fluorescence; Co(II) likely does not.

When considering all metals, Cu(II), Ni(II), Sn(II), Zn(II), and Co(II) all showed promising Thioflavin T results when compared to their respective controls (fig. 3).

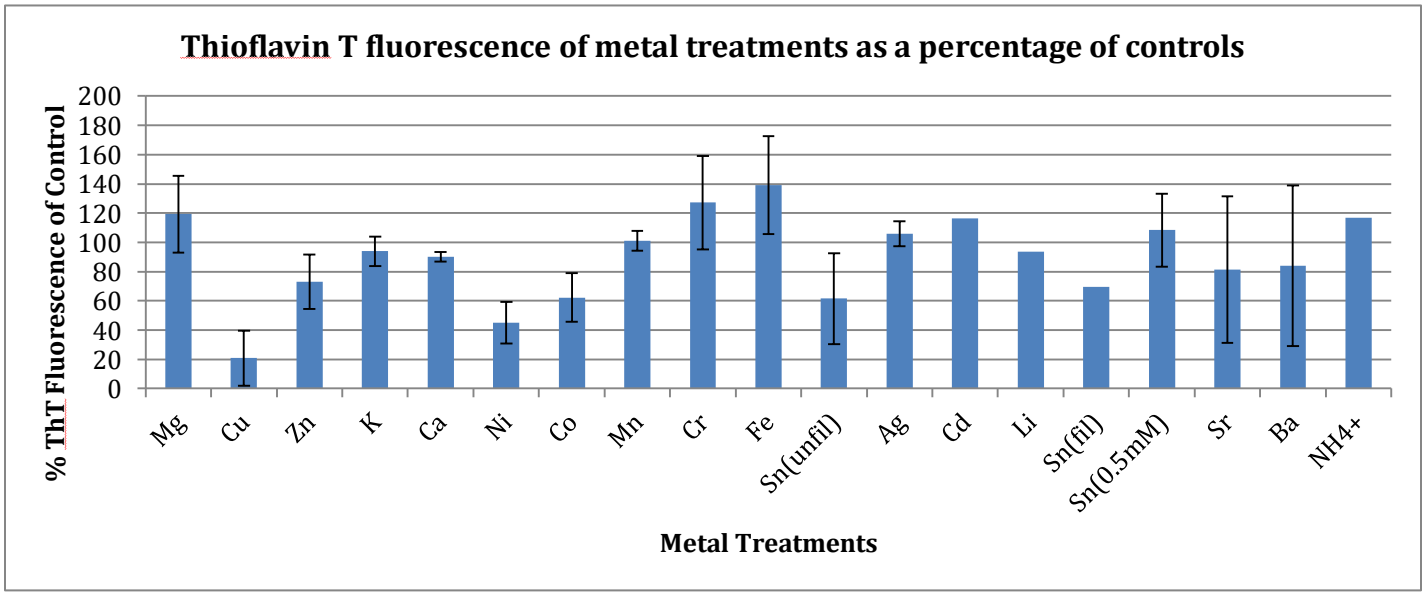

Figure 3: Max ThT fluorescence of various 10mM metal treatments (mostly divalent chlorides) as a percentage of their respective controls. Cu(II), Zn(II) Ni(II), Co(II), Sn(II) appear to inhibit ThT fluorescence.

AFM

All metals appeared to have more aggregated hIAPP than the control (Fig. 4, panel 1), especially Cu and Zn (Fig. 4, panels 2 and 3). Zn and Cu displayed the thickest fibers of hIAPP; Ni had dense but smaller fibrils (Fig. 4, panel 4) and Sn had thicker fibrils that were less dense (Fig. 4, panel 5).

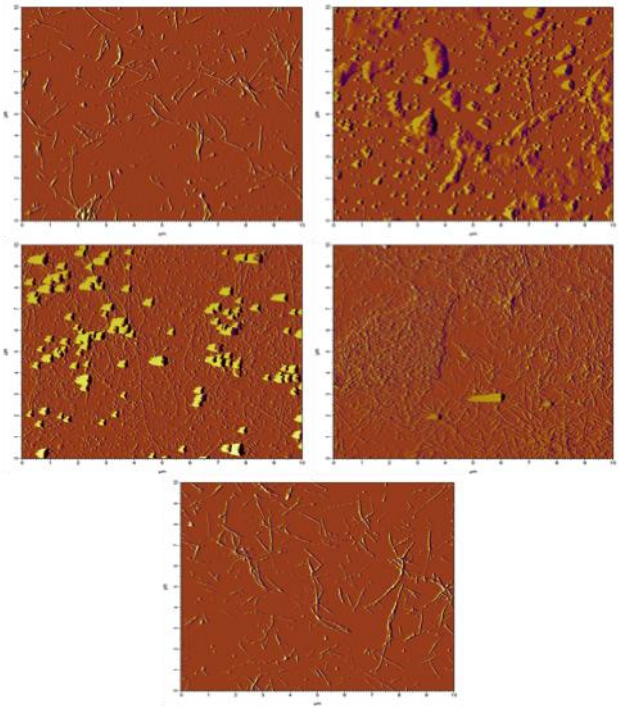

Figure 4: AFM pictures. From left to right, top to bottom,hIAPP control, Zn(II), Cu(II), Ni(II), Sn(II).

Discussion

This study set out to learn whether metal cations would be able to prevent the aggregation of hIAPP. The mechanism detailing Zn(II) inhibition of hIAPP aggregation has previously been elucidated (Brender *et al.*, 2010), and other studies have shown other divalent cations such as Cu(II) similarly inhibited Thioflavin T fluorescence. 19 positively charged species, including 13 metal dications and 5 metal monocations were tested, with 5 of the dications lowering Thioflavin T fluorescence compared to their hIAPP controls, including Zn(II). After examining the solutions of Zn(II), Cu(II), Ni(II), and Sn(II) under AFM, none appeared to decrease the thickness and density of fibril growth.

The common disagreement between the Thioflavin T fluorescence and AFM results may be due to interference by the metal with the dye binding hIAPP. In these instances, a low ThT fluorescence value would suggest the metal inhibited hIAPP aggregation when it really interfered with ThT binding aggregated IAPP. This has been shown to occur with

polyphenolics (Hudson *et al*., 2009), but not yet with metals. The metals tested herein should therefore be tested for their ThT-binding properties. Additionally, if the metals were to promote aggregation as Zn and Cu have been shown to do with $A\beta_{42}$ (Noy *et al*., 2008), fluorescence would decrease as the dye would not be able to bind as great a surface area. This illustrates the importance of AFM or Transmission Electron Microscopy (TEM) as a confirmatory test for suspected hIAPP aggregation inhibitors.

The discrepancy found between literature findings and the findings of this experiment may be explained by the metal ion concentration hIAPP was treated with, which in this experiment was 10 mM. Similar experiments used concentrations of 0.4 uM for (Ward *et al*., 2008) or 10-25 uM, more similar to physiological conditions in the Beta cells (Brender *et al*., 2010). In fact, Zn concentrations higher than 500 uM stop increasing the lag time (time before which hIAPP aggregates) and increase the elongation rate of fibrils, suggesting the mechanism for Zn interaction with hIAPP is different at lower and higher An concentrations (Brender *et al*., 2010). Since Cu has the same hypothesized His-18 binding mechanism as Zn, only stronger (Brender *et al*., 2010), it is possible the same trend is true for Cu. The only metal tested at different concentrations was Sn(II), as the chloride salt wasn't completely soluble at 10 mM; as such, 10 mM (unfiltered and filtered), 1 mM, and 0.5 mM concentration solutions were prepared and tested. However, the higher concentration supersaturated solution seemed to decrease ThT fluorescence best, indicating the trend for Zn is not true for at least Sn. This is possibly due to different mechanisms of interaction with hIAPP. Future work should attempt to discover optimal inhibitory concentrations for metal cations.

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