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Cell Toxicity Studies to Understand Neurodegenerative Parkinson's Disease

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ABSTRACT

Parkinson's disease is one of the most common neurodegenerative disorders. Parkinson's can affect all ages but is predominantly diagnosed in older people. The cause for Parkinson's disease is from the loss of dopamine as dopaminergic neurons are killed in the brain, causing symptoms that include shaky limbs, depression, and speech impediment. However, the mechanism for how the dopaminergic cells are progressively dying is unknown. In the study, SH-SY5Y, a dopaminergic cell line, was tested with MPP+, a compound thought to be most capable of causing Parkinson's. APP, an amyloid precursor protein, is a known toxin to SH-SY5Y, so this research tested the toxicity of MPP+APP within SH-SY5Y cells. The cells were exposed to different concentrations of MPP+APP and incubated for specific times and the cell viability was measured for cell by spectrophotometry. According to the data, MPP+APP is toxic to SH-SY5Y cells at concentrations higher than 400 micro molar and FFA is able to significantly inhibit MPP+APP beginning at 350 micro molar concentrations, with a 0.0010 p value. A test was also performed on Mn9D cells to evaluate the toxicity and inhibition of MPP+APP and FFA and the result showed significance in cell viability at 15 micro molar concentration with a p value of 0.00723

Keywords: *dopaminergic, MPP-APP, Parkinson's disease, SH-SY5Y.*

INTRODUCTION

Parkinson's disease (PD) is a neurological degenerative condition that slowly cripples the central nervous system of humans (Sapir 2014). PD affects spinal motor systems such as limb, gait, and respiratory functions, and also cranial motor systems involving facial definition, vocal articulation, and mastication (Chu 2015). This disease more commonly affects the older population, about 1.0%-1.5% in people 60 years or older (Sapir 2014). Other conditions accompanied with PD include dementia, intermittent delirium and hallucinosis (Tsai et al. 2014). There are two major subtypes of Parkinson's that exist. The first type is tremor-predominant common in younger people and progresses at a slow rate. The second type of Parkinson's is known as "postural imbalance and gait disorder" (PIGD). PIGD is more common in older people and has a much higher rate of progression in motor dysfunction than the first type of PD.

PD is indicated through the loss of dopaminergic neurons located in the substantia nigra pars compacta of the brain (Seidl et al. 2014). The death of these dopamine producing cells causes depression, slurred speech, and shaky movements in the limbs (Mayo Clinic). However, the mechanism explaining the loss of dopaminergic neurons is unknown (Rizza et al. 2015).

The accepted model in the progression of Parkinson's is a simple pathological process which results in the loss of pigmentation of the substantia nigra as dopaminergic cells progressively degenerate (Jose Obeso). As a result of the dopaminergic cells dying, the production of dopamine is greatly diminished. Some of the purposes for dopamine include the control of hormones released and being a neurotransmitter between neurons in the brain. The loss of function in these processes cause depression and motor deterioration as the concentration of dopamine decreases. Drugs such as Levodopa (L-Dopa) have been used to replace the lacking amount of dopamine produced, minimizing the effects of the disease. However, this treatment is temporary as symptoms such as dyskinesia, change in mental state, and reduced efficacy, gradually take over through a period of time, rendering the L-Dopa ineffective (Kopin Irwin). Because the model for the process and progression of Parkinson's disease is fairly simple, it was thought that a cure would be fairly simple as well. However, there are also affected non-dopaminergic transmitter systems that are related to PD which makes it a lot more complex (Jose Obeso).

Although we know the effects of Parkinson's disease, the reason for why the dopaminergic cells are progressively dying is unknown. L-Dopa is only a temporary treatment and does not target the center of the issue. One approach for analyzing the pathology of this disease focuses on MPP+ toxin and its effects on neurodegeneration. MPP+ has the closest result in symptoms of PD, so understanding how the toxin is killing the neurons may shed some light on the mechanism in question. My research is to determine how MPP+ is causing neurodegeneration and to find a prevention of the cell death. I exposed dopaminergic cells to different concentrations of the MPP+ toxin to observe the different levels of cell death. I then added the inhibitor, FFA, a compound

known to protect a different cell line, to observe its protection of the dopaminergic cells as well.

MATERIALS AND METHODS

The SH-SH5Y cells were grown using Petri dishes in Dulbecco's Modified Eagle Medium (DMEM). The media was removed from the Petri dish by aspirating with a vacuum while leaving the cells undisturbed on the plate. The dish was washed and aspirated twice with five milliliters of Phosphate Buffered Saline (PBS). To loosen the cells from the petri dish, Trypsin was added and allowed to stay on the cells for two minutes before being aspirated. The cells were then suspended in media and centrifuged for a minute. The supernatant liquid was removed and the pellet from the centrifuge was broken and the cells were re-suspended in eight milliliters of media and counted using a hemocytometer.

A portion of the above cell suspension was added to a new petri dish with more media to grow. The remaining cell suspension was appropriately diluted for the trials and 100.00 microliter aliquots were placed to each well of a 96-well plate. Before measuring the toxicity of the SH-SY5Y cells, the plates were incubated for two or three days until the media is darker orange than pink and the cells completely cover the bottom of the dish.

A series of concentrations was prepared from a 1mM stock solution of MPP-APP for the first trial (Table 1 Trial 1), and was added to the wells in the 96-well plates. After the toxin was added, the cells were incubated for 16 hours. Then, 10 micro-liters of the light sensitive compound, 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), was added to every well to nullify the effects of MPP-APP and incubated for two hours. MTT was also added to two free wells for a control. 210 micro-liters of Sodium Dodecyl Sulfate (SDS) were then pipetted into each well and 260 micro-liters were pipetted into the control wells and incubated again for four to six hours. 230 micro-liters from each well were then transferred into separate cuvettes and measured by spectrophotometry for absorbance values of the cell viability.

Table 1. MPP+APP Concentrations (µM).

TRIAL 1 SH-SY5Y	0, 5, 10, 15, 20, 25, 40, 50, 75, 100
TRIAL 2 SH-SY5Y	0, 100, 150, 200, 250, 300, 350, 400, 450, 500
TRIAL 3* SH-SY5Y	0, 250, 275, 300, 325, 350, 375, 400, 450
TRIAL 4* Mn9D	0, 5, 10, 15, 20, 25, 40, 50

*With FFA

A second trial was preformed using the same MPP-APP concentration values as mentioned above but the cells were incubated with the active toxin for 24 hours instead of 16 hours. Three more trials were performed using a higher MPP-APP concentration series, represented in Trial 2 of Table 1, and were incubated for 16 hours. After finding the p50 value, two more trials were performed with concentration values represented in Trial 3 of Table 1, along with placing an aliquot of FFA, a compound known to protect the Mn9D cell line, from the toxic effects of MPP-APP. A final trial was performed with the Mn9D cell line using MPP-APP concentrations illustrated in Trial 4 of Table 1, with one dish protected by the FFA compound and one without the FFA compound. All trials and plates were incubated at 37 ºC.

RESULTS

The first trial (Figure 1) uses concentrations of MPP+APP that are known to be toxic to Mn9D cells. However, as seen in the figure, no significant difference in toxicity is illustrated with a p value of 0.0747 between the control and 100µM. Cell viability in all treatments stayed above 90%. Another trial (not shown) incubated the cells in the same MPP-APP concentrations for 24 hours with similar results. Error bars are ±Standard Deviation (SD).

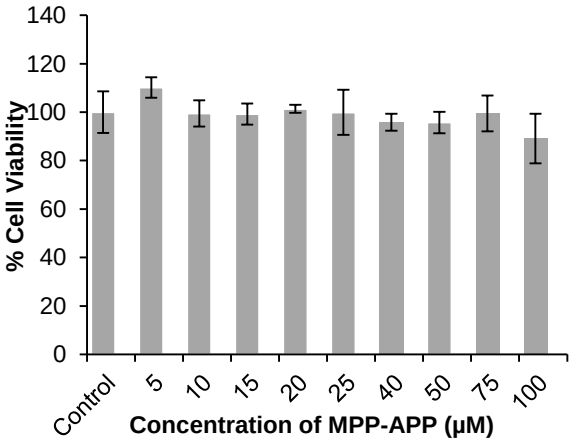

Figure 1. Toxicity of SH-SY5Y in MPP+APP in regular HCO3, 16 hours - 06/25/14

In Figure 2 MPP-APP concentration was increased and significant toxic effect on the SH-SY5Y cells is depicted as shown with 54% cell viability at 400 µM and 45% cell viability at 450 µM. Error bars are ±SD.

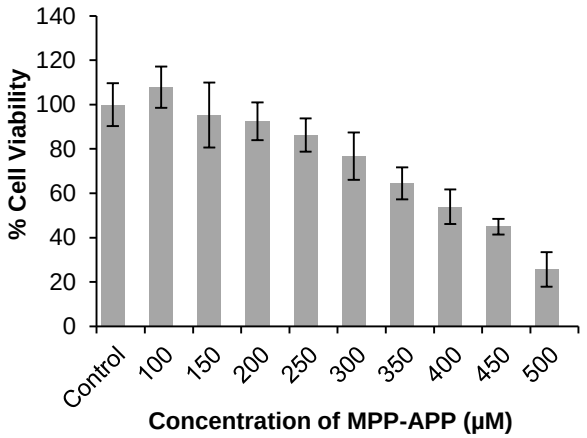

Figure 2. Toxicity of SH-SY5Y in MPP+APP in regular HCO3, 16 hours - 07/02/14

Based on the results from Figure 2, concentrations leading up the p 50 value were used with the addition of FFA. Cell viability in all concentrations, except for the control, stayed between 69% and 76%. Error bars are ±SD.

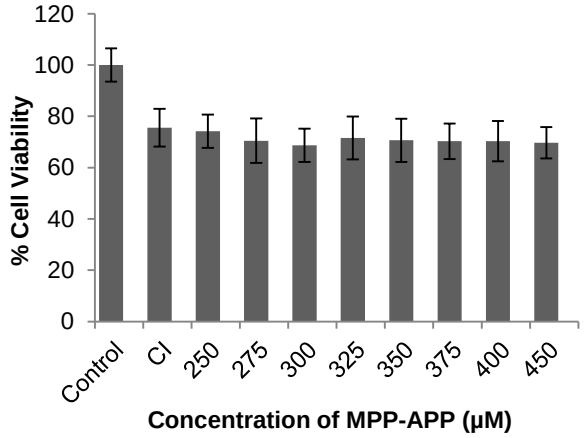

Figure 3. Toxicity of SH-SY5Y in MPP+APP in regular HCO3 with FFA, 16 hours – 08/02/14

Figure 4 is a comparison of cell viability in Mn9D Cells when exposed to MPP-APP and FFA. Using a t-test no significant difference is seen until 15 µM with a p value of 0.00723. Error Bars are ±SD.

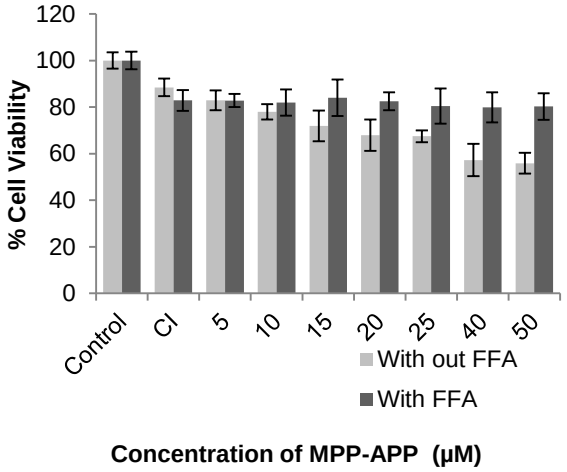

Figure 4. Comparison of Toxicity of Mn9D in MPP+APP with FFA inhibitor in regular HCO3.

DISCUSSION

Before the trials, it was hypothesized that the MPP-APP compound would be toxic to the SH-SY5Y cells. At the concentrations used in the first two trials, as is shown in Figure 1, MPP-APP was not initially toxic. At concentrations with proportional increases from 5µM to 100µM the cell viability never decreased below 90%. Samples were incubated for 24 hours in a second trial since there was no toxic effect in the first trial. However, there was no significant difference in toxicity as the cell viability still stayed above 90%. In a following trial (Figure 2), concentrations were increased fivefold, which showed that the MPP-APP is toxic to SH-SY5Y cells, as hypothesized. When concentrations started at 100µM and increased every 50µM up to 500µM (Figure 2), the cell viability decreased from 100% to approximately 25%. The results show that the MPP-APP compound is very toxic in concentrations from 400µM to 500µM but showed very little toxicity in lower concentrations.

The p 50 value, concentration of MPP-APP where cell viability is at 50 percent, is between the 400µM and 450 µM (Figure 2). I performed a trial with the inhibitor FFA, using MPP+APP concentrations leading up to the p 50 value, and excluding concentrations that had no significant toxicity (Figure 3). The test with added FFA showed that cell viability dropped to approximately 70%-75% in all the different toxin concentrations but stayed at a constant level when it was expected to consistently decrease cell percent viability (Figure 3). This shows that the FFA inhibitor compound does protect SH-SY5Y cells from high concentrations of MPP-APP.

Due to lack of time, a second trial was not performed to confirm that FFA is a good inhibitor from the MPP-APP compound in SH-SY5Y cells.

Even though it is known that FFA inhibits the toxicity of MPP+APP in Mn9D cells, I performed a trial in order to reproduce their results. Figure 4 confirms that the FFA compound is a good inhibitor for Mn9D cells when exposed to concentrations of the MPP-APP toxin from 5µM to 50µM with significance of inhibition at 15µM up to 50µM.

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