

The Effect of Melatonin on Tau Hyperphosphorylation in Hypothermic SH-SY5Y Cells

Purpose: This study was to prove that hypothermic conditions can be an in-vitro model of the tau hyperphosphorylation seen in Alzheimer's disease. Secondly, this study was an investigation of the viability of melatonin as a treatment for Alzheimer's disease, which can be applied to several other neurodegenerative disorders.

Introduction

A large branch of neurodegenerative diseases known as tauopathies, can be attributed to the abnormal hyperphosphorylation of the microtubule-stabilizing tau protein. Although there are several models Available for testing these diseases, such as mathematical and *in-vivo* models, there is no *in-vitro* model that has been shown to successfully cause the tau hyperphosphorylation of neurodegenerative diseases^[1]. Glucose deprivation has been a cause of pathological protein behavior in many diseases. Recently, studies have connected problems of hypothermia to glucose deprivation^[4]. This creates the possibility for using hypothermic conditions as a simulation of Alzheimer's disease in SH-SY5Y cells to produce an *in-vitro* model capable of furthering Alzheimer's research.

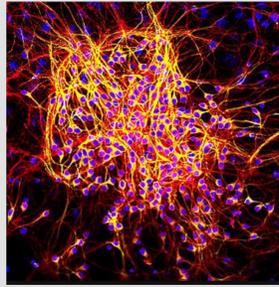


Figure 1: Stained hyperphosphorylated tau protein.

Alzheimer's disease

Alzheimer's disease (AD) is one of the fastest growing forms of dementia. There are currently more than 5 million Americans with Alzheimer's, and this is expected to increase to 16 million by 2050. The number of deaths from Alzheimer's disease has increased by 89% since 2000^[3]. Even with the current annual budget of \$214 billion dedicated to Alzheimer's research, only 5 drugs have been approved by the FDA for treatment, the most recent being approved in 2014^[2].

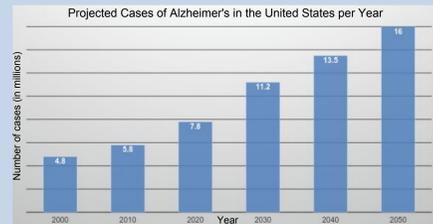


Figure 2: Recorded cases of Alzheimer's disease in the United States are predicted to continue increasing^[1].

There are two main hypotheses regarding the cause of AD: aggregation of the amyloid-beta ($A\beta$) protein and the hyperphosphorylation of the tau protein which leads to the formation of neurofibrillary tangles (NFTs)^[3]. Both rely on the pathological behavior of specific proteins and their role in cutting off interneuron communication^[4].

In 2016, a study on AD biomarkers concluded that melatonin, a hormone produced by the pineal glands central to maintaining circadian systems in the body, protected against the production of AD in aged mice^[4]. Due to the correlation between the tau protein and $A\beta$, melatonin could prove to have similar effects against tau.

Variables

- Negative Control: SH-SY5Y cells grown at 37°C with no melatonin; blank wells in ELISA
- Positive Control: SH-SY5Y cells grown at 30°C with no melatonin; standards in ELISA
- Experimental Groups: Dose response melatonin 1-50 μ M, time course 12/18/24 hrs
- Independent Variables: Concentration of melatonin added to cells and exposure time
- Dependent Variable: Tau Hyperphosphorylation at the pT181 epitope (as measured by ELISA analysis)

Abstract

Alzheimer's disease, among other Neurodegenerative diseases, is caused mainly by tau hyperphosphorylation. In this study, SH-SY5Y cells were grown in hypothermic temperatures (30°C) and compared against cells in prime growth conditions to determine tau hyperphosphorylation at each temperature. Melatonin was tested on hypothermic-induced hyperphosphorylated tau producing SH-SY5Y cells in varying concentrations for a dose response and time course analysis. Enzyme-linked immunosorbent assay (ELISA) was used to measure the expression of the pT181 isoform, a direct result of tau hyperphosphorylation. It was found that hypothermic incubation does increase tau hyperphosphorylation best at 18 hours of incubation, and melatonin only causes a decrease in isoform expression at 12 hours and with a high concentration. These results indicate that hypothermic conditions can serve as an in-vitro model for Alzheimer's research, but melatonin require a high concentration for a small effect.

Hypothesis

- Null Hypothesis (1): Hypothermic conditions will not induce tau hyperphosphorylation in SH-SY5Y cells.
- Alternative Hypothesis (1): Hypothermic conditions will induce tau hyperphosphorylation in SH-SY5Y cells.
- Null Hypothesis (2): Melatonin will not prevent or reverse tau hyperphosphorylation caused by hypothermic conditions in SH-SY5Y cells.
- Alternative Hypothesis (2): Melatonin will prevent or reverse tau hyperphosphorylation caused by hypothermic conditions in SH-SY5Y cells.

Materials

- SH-SY5Y Human Neuroblastoma Cells (ATCC)
- Ham's 12 MEM Growth Media (ATCC)
- Nature Made brand Melatonin (Target)
- Human Tau [pT181] phosphoELISA ELISA Kit (ThermoFisher Scientific)
- Cell Extraction Lysis Buffer (Thermofisher Scientific)

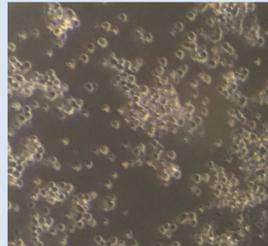


Figure 3: Premature SH-SY5Y cells under a microscope.

Methods

Cell Culture

- Cells were maintained in 1:1 Eagle's MEM and F12 Medium for one day
- Cells were seeded at 4.75×10^4 cells/mL in culture trays and incubated either at hypothermic 30°C or prime growth conditions at 37°C (experimental /control)
- Melatonin was administered both before hypothermic incubation to test preventative properties and after two hours of hypothermic incubation to test reversal properties

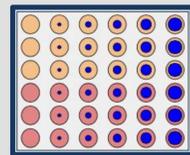


Figure 4: The layout of each experimental tray. Each column had different amounts of melatonin, from 0 to 50 μ M.



Figure 5: 96 well trays containing media, cells, and melatonin. Lighter color is due to pre-incubation.

Addition of Melatonin and Analysis

- Dose response test was conducted at 1 μ M, 5 μ M, 10 μ M, 25 μ M, and 50 μ M melatonin
- Time course was completed at 12, 18, and 24 hrs
- Trays were started so they were ready for analysis at the same time
- ELISA analysis was used to determine the hyperphosphorylation of all samples, each in triplicate

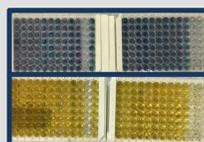


Figure 6: Preparation for ELISA, wells before and after addition of Stop Solution.

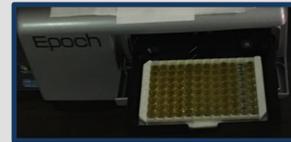


Figure 7: ELISA well plate in the 96 well plate reader.

Results

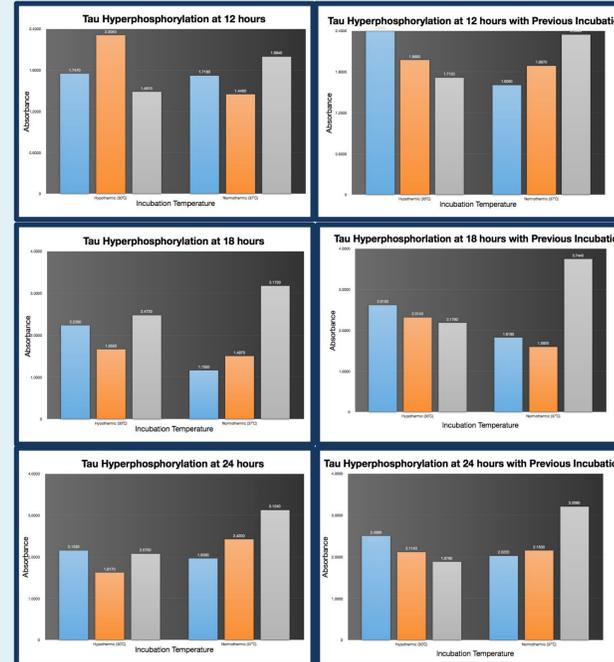


Figure 8: Comparison between hypothermic and normothermic incubation effects on tau hyperphosphorylation at various incubation times.

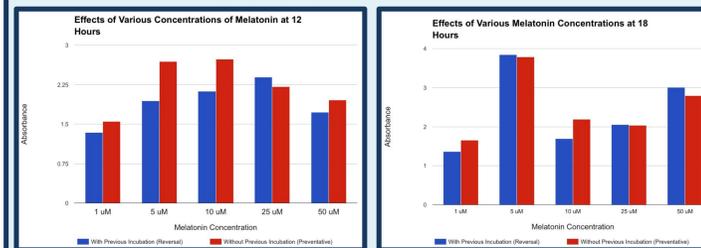


Figure 9: Comparison of reversal and preventative melatonin at different concentrations at 12 hours.

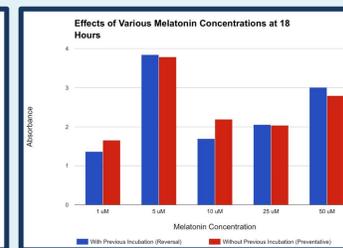


Figure 10: Comparison of reversal and preventative melatonin at different concentrations at 18 hours.

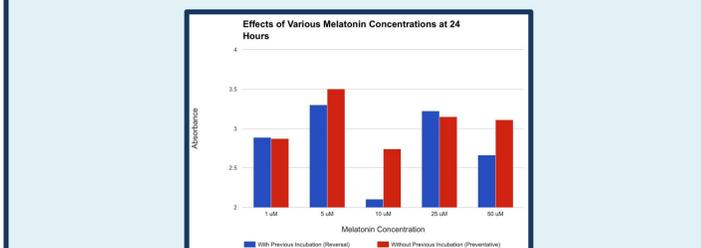


Figure 11: Comparison of reversal and preventative melatonin at different concentrations at 24 hours.

Discussion

It was found that hypothermic conditions can induce tau hyperphosphorylation slightly at 12 hours and more drastically at 18. However, by 24 hours of incubation the levels of tau hyperphosphorylation in the cells incubated at 30°C matched the levels of cells in the 37°C incubator. This can be attributed to the rapid cell growth and the glucose deprivation caused by a media lacking nutrients necessary for normal growth. 12 hours of incubation may not have been a suitable amount of time to induce the full hyperphosphorylation of tau. 18 hours had the largest difference of hyperphosphorylation likely due to the age of the media and the amount of time in the hypothermic conditions. Therefore, for the first hypothesis, the alternative can be accepted at 18 hours of incubation.

Melatonin proved to reduce tau hyperphosphorylation at the 12 hour incubation only, and best at high concentrations. Preventative melatonin (when administered without previous hypothermic incubation) began to work at a lower concentration than reversal melatonin. At 18 and 24 hours of exposure, the melatonin did not have a significant effect. This shows that the effects of hypothermic incubation are stronger than the effects of melatonin, proving it to only be effective in high concentrations at early stages of hyperphosphorylation. Therefore the alternative hypothesis can be qualified to only apply to cells with 12 hours of incubation and high concentrations of melatonin.

Conclusion

Hypothermic incubation induces tau hyperphosphorylation best at 18 hours of incubation. This length and temperature of incubation can serve as an *in-vitro* model for Alzheimer's disease among many other neurodegenerative diseases. Additionally, melatonin can reduce and prevent pathological behavior of tau only at high concentrations and early stages of hyperphosphorylation. At other incubation times and lower concentrations the effects of hypothermic incubation are stronger than the effects of melatonin, making its effects insignificant.

Further work

Further experimentation would be done to establish tau hyperphosphorylation with cell degeneration. The addition of Fluoro-jade B staining will indicate cell degeneration and apoptosis due to hyperphosphorylation. Additionally, this experimental study would benefit from running a larger number of samples at both 12 and 18 hours of incubation, as these were the incubation times with the most notable differences.

Acknowledgements

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References

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