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Laura Houghton

Abstract

Importance: Parkinson's disease is only one of several conditions classified under Lewy Body Disease, which is characterized by the build-up of the alpha-synuclein protein in neuronal cells. This causes cellular dysfunction and ultimately, cell death. As such, research on this protein is both critical and ongoing.

Objective: The purpose of this study was to explore the effects of melatonin and catalase supplements on alpha-synuclein-expressing Saccharomyces cerevisiae cells under oxidative stress created by adding H2O2.

Design: A mixed-methods research approach and experimental research method were used to investigate changes in cell growth, survival, and morphology.

Results: It was determined that the supplements do not have any significant effect on either cell growth or morphology and that melatonin helps to reduce cell death in more severe conditions of oxidative stress.

Conclusion: This effect is most likely because of melatonin's dual ability to both detoxify reactive oxygen species and initiate increased production of antioxidant enzymes like catalase.

Keywords: oxidative stress, melatonin, catalase, *S. cerevisiae*, α-synuclein, hydrogen peroxide

Literature Review

Introduction

Across the world, more than 50 million people are currently suffering from a neurodegenerative disease.[1] Alzheimer's Disease (AD) is the most common, followed by Parkinson's Disease (PD) and Dementia with Lewy Bodies (DLB).[2] All three are progressive conditions,[2] and PD and DLB are both considered to be forms of Lewy Body disease.[3] DLB begins with cognitive symptoms, such as loss of memory, but progresses later to more physical symptoms. PD, on the other hand, is generally more of a movement disorder leading, most notably, to tremors and muscle rigidity; although it sometimes progress to PD dementia, which includes more cognitive symptoms.[4] The Lewy Body disease that underlies them both is characterized by the build-up of protein clumps, known as aggregates or Lewy Bodies, in the cytoplasmic regions of neurons.[2] Such aggregates inhibit cellular communication and neuronal functioning, leading to the symptoms discussed above. The protein alpha-synuclein has been shown to be the main component of these Lewy Bodies[5] and has thus been the focus of much research over the past couple decades. Despite this, researchers are still working to unlock the specific factors that cause it to form aggregates as well as different molecules and conditions that could prevent or reverse this aggregation.

Alpha-synuclein

The alpha-synuclein protein is found in many parts of the cell, including the cytoplasm, synaptic vesicles, the plasma membrane, lysosomes, lipid rafts, and nuclei.[6] It is generally agreed that alpha-synuclein is most likely a regulatory protein involved in several different necessary processes, including "neurotransmitter release [and] vesicle trafficking," among others.[2] It contains 140 amino acids,[7] and its behavior and functioning can be affected by many different factors, including oxidative stress.[8]

Overview of oxidative stress

Oxidative stress is a cellular condition created by the imbalance of reactive oxygen species (ROS) and the cell's various antioxidant defenses.[9] Superoxide and hydroxyl radicals are the most common examples of ROS,[10] and are produced by the mitochondria during aerobic cellular respiration.[10] Hydrogen peroxide (H2O2) can also be included in this group, but that categorization is slightly more nuanced because H2O2 is a byproduct of the breakdown of superoxide ions by the enzyme superoxide dismutase, rather than a molecule produced directly by the mitochondria.[11] Regardless, it is still capable of causing immense cellular damage. Typically, the production of ROS is balanced by antioxidant defenses within a cell, which include both enzymatic and non-enzymatic components.[12] Non-enzymatic antioxidants can include both endogenous molecules, which originate inside the cells, and exogenous molecules, which originate outside the cells.[9] Enzymatic antioxidants most commonly include superoxide dismutase[8] and catalase,[11] which work to detoxify and/or destroy ROS. When balance is maintained, ROS play an important role as '2nd messengers,' activating cellular signal transduction pathways, facilitating calcium signaling and the actions of growth factors and cytokines.[13] Since this type of cellular communication is crucial to the growth and proper functioning of cells, the complete eradication of ROS would not be an ideal solution.

Oxidative stress as an agent of disease

Most studies show a connection between oxidative stress and cellular damage. The ROS imbalance leaves proteins, lipids, DNA, and other macromolecules more vulnerable to oxidative modification, which affects their structure and can eventually lead to cell death.[14] One potential problem specifically with protein modification is protein aggregation, such as that observed in both LBD and PD. A 2019 article by Lévy, et al. presented a clear evaluation of the link between oxidative stress and protein aggregation by analyzing several different studies that tested it. They concluded that oxidative stress can be both a cause and result of protein aggregation and that the misfolding of proteins, which can be caused by oxidative stress, makes them significantly more prone to aggregation.[15] The concept of a connection between oxidative stress, protein modification, and protein aggregation is not a new one, though. In 2003, Ischiropoulos and Beckman came to a similar conclusion. Through their analysis of multiple studies, they gave credence to the idea that oxidative stress can cause the nitration of proteins and noted that, in a variety of diseases, including DLB and AD, the proteins built up in neurons have been nitrated at much higher rates than normal.[14] They also emphasized the plausibility of this link between oxidative stress and neurodegenerative diseases by noting the high susceptibility of neurons to oxidative stress.[14]

Mitochondrial theory of aging

Even with such a connection, the tie between all of these concepts still seems to be missing. Since the diseases discussed above are predominantly found in older populations, that tie must involve aging; more specifically, the mitochondrial theory of aging.[16] This theory states that mitochondria become less functional as time passes, in large part because excess ROS that have not been cleared away continue to build up over time and cause damage to the mitochondria.[16] This is a vicious cycle because damage to the mitochondria can cause increased production of ROS and a decreased ability to detoxify them, which leads to even more damage,[16] indicating that increasing antioxidant defenses could help to curb oxidative stress and its damaging effects.

Melatonin

One such antioxidant defense is melatonin, which is present in all organisms that perform aerobic respi-

ration and is both chronobiotic and cytoprotective.[17] This means that it can both modify the phase and amplitude of biological rhythms and reduce mild inflammation, which is why it is commonly used to help treat sleep disorders and is of interest as an antioxidant.[17] In cells facing oxidative stress, melatonin can help by both working to detoxify ROS and by stimulating the production of antioxidant enzymes, such as catalase and superoxide dismutase.[18] A 2017 study by Vázquez, et al. examined the effects of melatonin on the yeast species *Saccharomyces cerevisiae* when exposed to oxidative stress and found that melatonin mitigated cell damage by decreasing the accumulation of ROS and helped prolong the up-regulation of genes related to antioxidant defenses.[18] Additionally, many studies over the past few decades have investigated melatonin in the context of protein aggregation, including one by Lahiri in 1999, which concluded that melatonin helps prevent the development of the amyloid precursor protein, which contributes to protein aggregation in patients with AD.[19]

Catalase

As mentioned earlier, catalase is one of the body's most important antioxidant enzymes. Its job is to break down H2O2 into oxygen and water,[11] which it can do extremely efficiently.[20] Like melatonin, it is found in nearly all organisms that perform aerobic respiration.[11] Because of its strong antioxidant properties, catalase, like melatonin, has been highly investigated in the context of neurodegenerative diseases thought to develop partially due to oxidative stress. It has also been hypothesized that a lack or decreased functioning of catalase can contribute to the formation of those diseases.[11] Martins and English studied these ideas in 2013 by adding H2O2 to different knockout Saccharomyces cerevisiae strains in both YPD media and nutrient-free buffer and concluded that expression of the CCT1 gene, which codes for catalase, was essential for cell survival but was only fully functional in the nutrient-rich media.[20] While studies like this advance the claim that catalase is extremely important in antioxidant defenses, some studies suggest otherwise. A 2001 study by Ilizarov, et al. found that the supplementation of manganese superoxide dismutase had a significantly greater impact on the survival of cells under oxidative stress than did catalase and suggested that the breakdown of superoxide anions, not H2O2, was the rate-limiting step.[21] Thus, a debate exists about whether or not catalase supplementation has a significant effect on cell survival.

Research gap and question to be addressed

Despite the wide variety of studies that have been done on both melatonin and catalase, there has been a lack of studies comparing the two within the same experiment. Additionally, the protective effects of melatonin against damage by oxidative stress and protein aggregation have yet to be studied together, and there have been very few investigations into the effects of catalase on protein aggregation. It is imperative that these gaps be addressed, however, because "a better understanding of the role of oxidants in neurodegeneration," along with factors that may mitigate them, "still holds a largely unfulfilled potential to reduce the burden of both acute and chronic neurodegeneration."[14] This leads to the research question: To what extent do melatonin and catalase supplements affect the survival of alpha-synuclein-expressing *Saccharomyces cerevisiae* cells when exposed to oxidative stress? It was hypothesized that under this condition, catalase would have a greater protective effect than melatonin because of catalase's direct role as an antioxidant enzyme versus melatonin's less direct role as a promoter of enzyme production. The main purpose of this experiment was to combine several ideas that had previously been expressed independently of each other, as well as to encourage the continuation of research into both melatonin and catalase.

Methods

Research approach

This study used a mixed methods approach to quantify cell survival under different levels of oxidative stress, as well as to observe the cells for changes in size and shape. This allowed for direct comparison of the experimental groups by putting them all on the same scale and removing ambiguity and subjectivity. Additionally, it allowed for visual observations of changes in shape, which can be difficult to quantify.

Almost all studies in this field use quantitative data,[2-3,5-7,8,18-20] and several others make use of both quantitative and qualitative data.[3,21]

Research method

An experimental research method, performed entirely in a lab, with the use of cell cultures, was employed to obtain both the qualitative and quantitative data. Using a laboratory setting increased control over the conditions to help keep variables like temperature more constant. This study did not have the capability to run human trials, so a model organism was used instead to make the study more feasible. Many studies, such as those by Hodara, et al. and Weston, et al., utilized multicellular organisms in their studies (mice and zebrafish, respectively); however, certain limitations, including a lack of resources and experience, prevented the use of such organisms in this study.[2,7] Fortunately, though, the use of cell cultures has been well established as a reputable method for experimentation in this field and has been used by a plethora of different studies.[3,6,18,20,22]

Subject selection

The yeast species *Saccharomyces cerevisiae* was used in this study to examine the effects of catalase and melatonin supplements on oxidative stress-induced cell death. This species was selected for several reasons, including practicality. Yeast cell cultures are relatively easy and inexpensive to care for, and they grow quickly.[23] More importantly, though, S. cerevisiae has been established by many studies as an appropriate model organism for studying Parkinson's disease and alpha-synuclein.[5,22-23] The specific strain used in this study was w303 Δhsp104 303GAL-a-syn-YFP;304GAL-a-syn-YFP, modified to express the alpha-synuclein protein and gifted generously by the Jackrel lab at Washington University.

Protocol

The following protocol, adapted from a 2019 article by Tran and Green,[24] was used to obtain data.

Both liquid and solid yeast media were made according to a recipe from the Grimes Lab at the University of Delaware (Appendix A), and the solid media was distributed equally among 40 sterile plates. A single yeast colony from the starter plate was removed with a sterile inoculating loop into 100μL of liquid media and resuspended by pipetting. This was then added to 149.9mL of liquid media and left to grow in a shaking incubator at 30°C and 220 rpm until it reached an OD600 value of 0.4-0.5 (approximately 24-26 hours).

40mL of yeast cell culture were placed in a 100mL flask and labeled as Group A. 108mL of the remaining cell culture were induced with 12mL of a sterile 20% galactose solution and incubated for 4 hours at 30°C and 220 rpm. The 120mL of induced culture was divided evenly into three 100mL flasks, labeled as Groups B, C, and D, respectively. A 5mM catalase supplement (0.017g) was applied to Group C, and a 5mM melatonin supplement (0.046g) was applied to Group D. The 10mL volumetric flasks and graduated cylinders were labeled, and H2O2, followed by yeast/media solution from the appropriate 100mL flasks, was added to each one to achieve a total volume of 10mL and the specified concentration (Appendix B). The samples were incubated at 30°C and 220 rpm for 30 minutes.

1mL was removed from each sample, placed into an appropriately labeled 1.5mL microcentrifuge tube, and centrifuged for 2 minutes at 12,200 rpm. Supernatants were removed, and cell pellets were resuspended in 1mL distilled water. A 96-well plate was used to create a dilution series of each experimental group, with the rows, from top to bottom, representing the dilutions 100%, 10%, 1%, 0.1%, 0.01%, and 0.0001%. One column was used for each of the twelve groups. Next, 200μL from the appropriate microcentrifuge tube was added to each of the wells in row A, and 20μL from each of these wells was added to 180μL of distilled water in row B and mixed with a pipette. This was then repeated for rows C-F. Three media plates were labeled for each experimental condition. On each plate, 5μL was added from each well in the appropriate column, going clockwise in a circle around the plate. After drying, the plates were inverted and stored at 30°C, and for the following five days, pictures were taken of each plate.

Protein isolation and western blot assays were performed for each experimental group, according to protocol from both Edvotek and Cell Signaling Technology. Buffers, gels, membranes, and antibodies were

generously provided by both companies. Since these assays did not yield any results, a more detailed protocol has been omitted.

Yeast cells from each subgroup were resuspended in 100μL distilled water, and 10μL was placed on a slide, stained with 2μL methylene blue, and visualized for shape, size, and viability.25 Three pictures were taken for each of the microscope slides, and a 36-square grid was laid over each picture. In order to acquire numerical data, six boxes were counted for each picture, meaning eighteen boxes total were counted for each experimental condition. The number of alive and dead cells was recorded for each of these, and a ratio of dead to alive cells was calculated. These ratios were averaged for each condition, and standard errors were calculated. These average ratios were plotted on graphs in various combinations to visualize relevant results.

Validity

Internal validity was ensured through the use of control samples and a relatively constant environment. Control samples were maintained throughout the experiment as uninduced samples that did not receive any supplements. Since these cells were not induced to begin producing alpha-synuclein, they were not expected to show any results for the western blot, therefore making them an effective negative control. To prevent contamination, the yeast media, as well as all glassware used, was autoclaved prior to use, and all steps up until the western blot, with the exception of incubation, were performed in a biosafety cabinet. Materials such as serological pipettes, micropipette tips, and microcentrifuge tubes were all sterile and unopened prior to use. Additionally, the use of methylene blue stain, which only enters dead cells, for visualization provided criterion validity.[25]

Limitations

Unfortunately, this study had some limitations. The most substantial limitation was the fact that a high school lab was used. While this setting did allow for the regulation of most environmental factors, it fell short in a few areas, including humidity control. Additionally, some professional-grade equipment that would have helped add more control to the study, such as a western blot transfer apparatus, was not available. This apparatus is typically used in western blotting to transfer proteins from the PAGE gel to the nitrocellulose membrane with the use of an electric current and adds consistency because the same amount of current can be applied to each transfer. The lack of this device could have been one of the reasons why the assay performed in this study was unsuccessful.

Results

Summary

The purpose of this study was to investigate the impacts of melatonin and catalase supplements on the growth and survival of alpha-synuclein-expressing *S. cerevisiae* cells under oxidative stress. Cells were grown on media plates in a dilution series, and their growth over a period of five days was observed. Samples from the plates were stained with methylene blue and visualized at 1000x optical zoom. The numbers of dead and alive cells were counted, and a ratio was calculated to provide quantitative data. The main findings were as follows:

1. Uninduced cells generally had more growth than induced cells.
2. Neither the addition of a supplement, nor the addition of oxidative stress had any observable effect on cell morphology.
3. In general, increased oxidative stress, as measured by H2O2 concentration, corresponded to higher percentages of dead cells.
4. Neither catalase nor melatonin had a statistically significant effect on cell death when oxidative stress was not present.
5. At higher levels of oxidative stress, melatonin, but not catalase, lead to a statistically significant decrease in cell death.

Uninduced vs. induced cells

Of the four treatment groups, Group A, the control, was the only group that was not induced by galactose exposure, which means the cells in this group should not have been expressing alpha-synuclein. Based on

Table. Largest Dilution Factor at which Growth was Observed After 5 Days													
H2O2 Concentration (mM)	A - Uninduced, No supplement			B - Induced, No supplement			C - Induced, Catalase supplement			D - Induced, Melatonin supplement			Mode
0	104	103	104	103	104	104	104	103	103	104	104	103	104
2	104	104	104	103	104	103	104	103	103	103	104	103	104/103
4	104	103	103	102	103	103	103	104	104	103	103	104	103
Mode	104			103			103			103			

visual observations, none of the groups showed any growth on the first day after being plated. On the second day, though, a difference in growth was seen between Group A and the rest of the treatment groups. Of the nine plates (three for each H2O2 concentration) from Group A, eight showed growth. In comparison, only three plates from Group B, two plates from Group C, and two plates from Group D showed growth. A slight difference was also observed between Group A and the rest of the groups in terms of the largest dilution factor at which growth was observed after the five-day observation period.

This table shows the largest dilution factor at which cell growth was observed after five days for each of the thirty-six plates. Across all three H2O2 concentrations, growth was observed most frequently at the 104-dilution factor for Group A, but for all of the non-induced groups, growth was observed most frequently at the 103-dilution factor.

Cell morphology

Based on visual observation of the cells at 1000x magnification, there were no noticeable differences in cellular morphology between the four main treatment groups and the different H2O2 concentrations. The cells observed were generally circular in shape, and while there appeared to be some slight variations in cell size within each sample, a consistent difference between groups was not observed.

Figure 1. 1000x Microscope Images from Each of the 12 Experimental Conditions

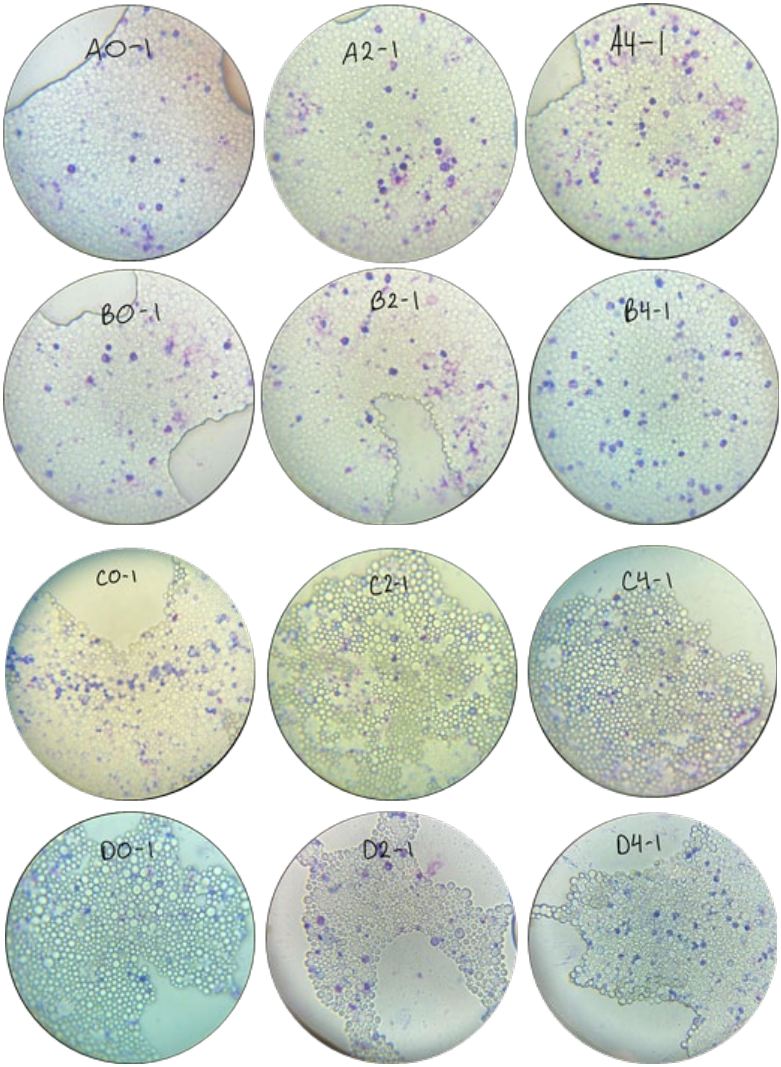
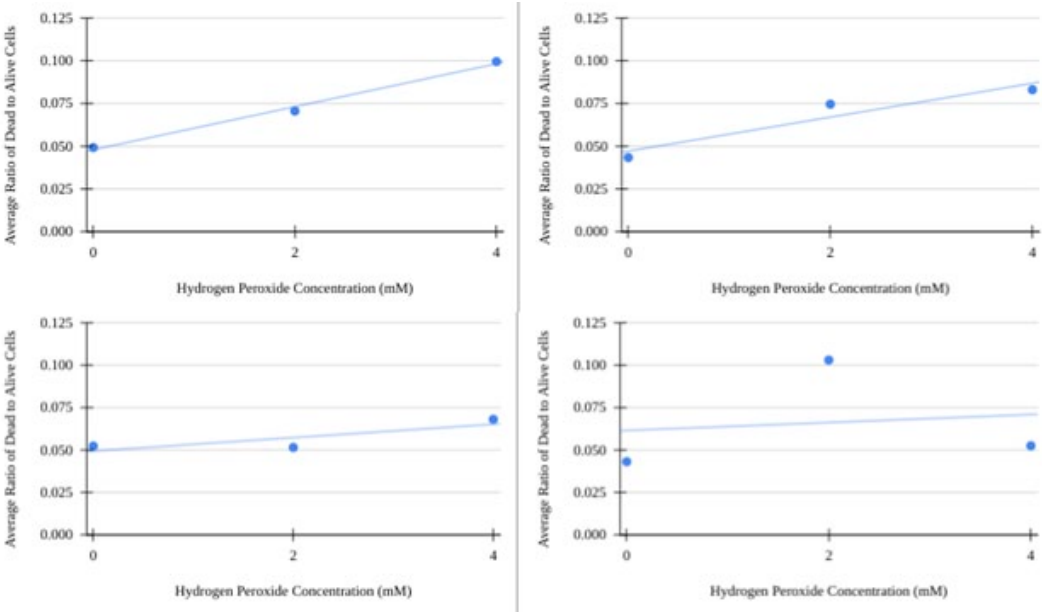


Figure 1 shows one of three images taken for each of the twelve experimental conditions (three different H2O2 concentrations for each of the four main treatment groups). As stated above, no consistent morphological differences between the groups were observable.

Oxidative stress and cell growth

Overall, across all four treatment groups, a higher level of oxidative stress, as represented by a higher concentration of H2O2, corresponded to a greater ratio of dead to alive cells. Any cells that appeared blue/purple were counted as dead because of the entrance

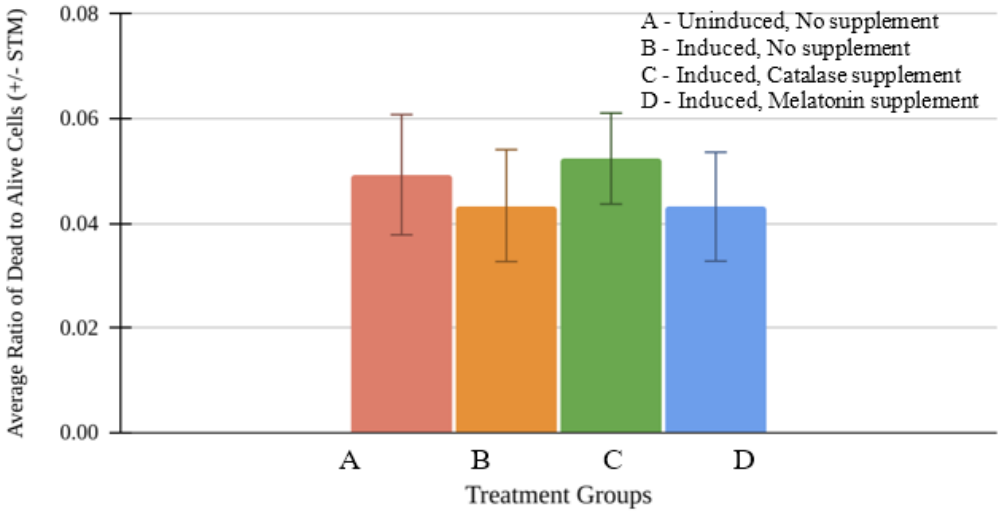
Figure 2. Effects of H2O2 Concentration on the Ratio of Dead to Alive Cells



of the methylene blue stain, and any cells that appeared white were counted as alive.

Figure 2 shows four different graphs, one for each of the four main treatment groups, indicated by the letter labels in the top left corners. The points plotted for each of the three H2O2 concentrations indicate the averages of the eighteen total boxes counted from the microscope pictures for that concentration, and the lines represent the linear lines of best fit. In each of the four graphs, it can be noted that the slopes of these lines are positive, indicating a positive correlation between increased oxidative stress and increased cell death.

Figure 3. Effects of Melatonin and Catalase Supplements on Yeast Cell Death at 0mM H2O2



Supplements in the Absence of Oxidative Stress

Focusing in on the 0mM concentration, which represents a lack of oxidative stress, it was observed that neither melatonin nor catalase supplements affected the ratio of dead to alive cells.

Figure 3 shows the ratio of dead to alive cells at 0mM H2O2, along with the standard error for each, which was calculated by averaging the cell counts from each microscope view. There is no discernable pattern in the ratios of cell death between these four treatment groups, meaning that the supplements did not have an effect in this condition.

Supplements in the Presence of Oxidative Stress

Conversely, at the 4mM concentration, the highest H2O2 concentration tested, the results indicated a statistically significant difference between treatment groups B and D, the induced cells with no supplement and the induced cells that received the melatonin supplement.

Figure 4 is very similar to Figure 3, with the only major difference being that it shows the cell survival at 4mM H2O2, instead of 0mM. Compared with Group B (Induced, No supplement), Group C (Induced, Catalase supplement) did not show a statistically significant decrease in cell death ($p=0.2278$); however, Group D (Induced, Melatonin supplement) did show a statistically significant decrease ($p=0.0284$).

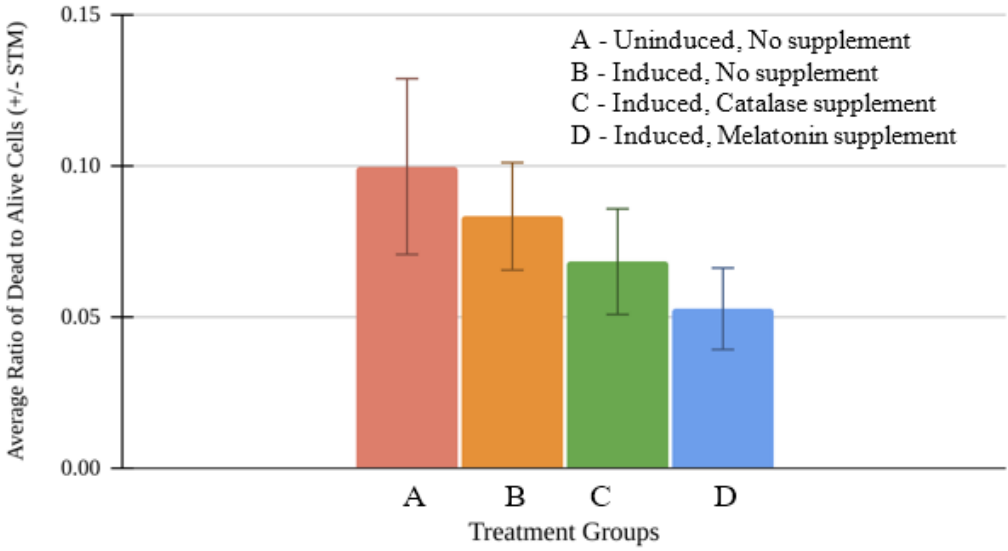
Discussion

Significance

One major implication of this research is that its preliminary findings line up with several ideas in the surrounding literature. For instance, as discussed in the literature review, oxidative stress has been heavily linked to cellular damage and as a result, cell death.[9-14,16] The results of this study, which showed a trend of increasing cell death as H2O2 concentration increased, support this idea. Additionally, multiple studies had already suggested that melatonin has protective effects against oxidative stress,[17-18] and this study, which showed that melatonin helps reduce cell death at 4mM H2O2, provides additional evidence for this.

This study also found, however, that neither mela-

Figure 4. Effects of Melatonin and Catalase Supplements on Yeast Cell Death at 4mM H2O2



tonin nor catalase have an impact on cell survival at 0mM H2O2, an idea which is supported by the role of both of these supplements in the body. Since they are both important for antioxidant responses,[13] it follows that in the absence of oxidative stress, they would tend to be present at lower levels within the cells, and even if supplemented in excess, they would not have much of a function to perform. In the case of catalase, H2O2 is needed as a substrate, so if this substrate is not present, no reaction will take place. Similarly, one of melatonin's functions is to detoxify ROS, but if ROS are not present, it cannot perform this function. Thus, at low H2O2 concentrations, neither supplement is able to have an impact.

While it was initially hypothesized that catalase would have a greater effect on cell death because of its more central role in the cellular oxidative stress response, the results supported the opposite idea, showing that melatonin supplementation leads to a decrease in cell death at higher levels of oxidative stress, while catalase does not. One potential reason for this is melatonin's ability to both induce the production of more antioxidant enzymes and also directly detoxify ROS.18 While it is not as intrinsically involved in the cell's natural antioxidant response as catalase, these results suggests that its dual action may allow it to have a greater impact.

Alternative explanations

Additionally, there are a few alternative explanations that could relate to the conclusions of the present study. The first, and most significant, is that the concentrations of melatonin and catalase added to the samples were not optimal. As determined by a 2017 article by Vázquez, et al., 5μM would have yielded the most favorable results for melatonin.[18] Unfortunately, because of limitations in laboratory equipment, a 5mM concentration had to be used instead, with the theory that any effects would be heighted and thus, easier to observe with limited technology. It cannot be overlooked, though, that this concentration may have been too high and could have caused some adverse effects. Also, a lack of previous studies focusing on catalase meant that dosing guidelines for catalase were not available. The amount of catalase that was added was intended to be roughly the same concentration as the melatonin, but this was most likely not the ideal concentration to use. The fact that neither of these supplements were in their optimal concentra-

tion range could be a reason for the observed difference in effect between the two.

Another fact to keep in mind is that catalase is somewhat of a secondary enzyme in the antioxidant process, as it breaks down H2O2, instead of superoxide ions, for instance.[20] This should not have had much impact on the actual experimental results because the oxidative stress was created with H2O2, not superoxide ions; however, it could diminish the applicability of the results in a more complete disease model.

Limitations

Despite the controls that were set in place for this experiment, the results do have several limitations. For starters, the sample size was very small. Due to both time and monetary constraints, only one experimental replicate was able to be performed. This means that any potential errors and/or outliers present throughout the experiment may not have been able to be identified and accounted for. It also means that, while the p-value was low for melatonin in this experiment, there was really not enough data for this value to hold much weight without further testing. Additionally, the method used for collection of quantitative data relied on human observation and counting, which is inherently error prone. The boundaries between the different cells were not always as clear as would have been ideal, so over or under counting could have been a factor in the results. For example, the average ratio of dead to alive cells for Group D at 2mM H2O2 (as shown in Figure 2) did not fit the trend shown by the linear line of best fit. This could be due to several reasons, but inconsistencies in counting as a result of human error was determined to be the most likely cause. As such, this point was considered to be an outlier in the data.

Finally, the most significant limitation in this experiment was the fact that protein expression could not be verified in the induced cell groups. This verification would have come through the western blot, but as that assay did not yield results, there is no such verification. Thus, there are two ways to interpret the results. One is as described above, with the assumption that the induced cells were expressing alpha-synuclein, while the other operates under the assumption that none of the cells were expressing alpha-synuclein, thus making groups A and B functionally the same. This view does not invalidate the experiment's results, though, as it does not take away the protective effect demonstrated by melatonin; it only removes the alpha-synuclein component.

Further research

The results of this study are neither entirely conclusive nor all-encompassing, which means that much more research in this area is needed in order to properly understand these supplements and their effects. First off, a replication of this experiment performed in triplicate is needed to yield results that can truly be quantified. Also, since the western blot in this experiment did not yield results, a replication of the experiment in more ideal conditions that give the western blot a greater chance of success would be highly beneficial. Additionally, future research should include a wider range of H2O2 and supplement concentrations tested. Doing so would not only enable researchers to see the effects of these supplements at a greater variety of oxidative stress levels, but also to see how increasing or decreasing the amount of supplement added could amplify their positive effects and/or introduce negative ones. Finally, future studies could also change the time of supplement administration by pretreating the cells instead of applying the supplements at the same time as the H2O2.

Research like this will provide better insight into the ways in which both of these supplements work in cells, as well as whether or not they may truly be effective in helping cells recover from oxidative stress. If this is found to be the case, this research will also begin to shape ideas of appropriate and effective doses, and the same principles may be able to be expanded to other diseases that are also linked to mitochondrial aging, oxidative stress, and/or protein aggregation, such as Alzheimer's disease, Huntington's disease, and frontotemporal lobar degeneration, among others,[1] providing novel therapies to help slow, or reduce the effects of, their progression.

Conclusion

The results of this study did not support the initial hypothesis that the introduction of a catalase supplement would have a greater protective effect than a melatonin supplement on yeast cells under oxidative stress. In fact, the results showed the opposite, most likely due to melatonin's dual antioxidant capabilities. The study did fill a gap, though, in the direct comparison of catalase and melatonin, which is important because of their similar functions in the body. Additionally, this study aimed to fill the gap for both melatonin and catalase with regards to their effects on alpha-synuclein aggregation under oxidative stress; however, this question was not successfully answered because of the failed western blot, meaning that further studies are needed to reach a conclusion. Regardless, this study supports the idea of using melatonin supplements to help mitigate the effects of diseases linked to oxidative stress, such as Parkinson's disease and Dementia with Lewy Bodies; although extensive dosage and efficacy testing are still necessary.

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Appendix A

Liquid yeast media (2 liters)

- 1.6 liters of distilled water
- 13.4 grams of a yeast nitrogen base without amino acids (generously provided by the Grimes lab at the University of Delaware)
- 1.74 g CSM-His powder (Sunrise Science #1006-010)
- 400 mL of 10% sterile filtered Raffinose solution (added after autoclaving)

Solid yeast media (1 liter)

- 0.8 liters of distilled water
- 6.7 grams of a yeast nitrogen base without amino acids
- 0.87 g CSM-His powder
- 20 grams of agar
- 200 mL of 10% sterile filtered Raffinose solution (added after autoclaving)

Note: This media was distributed into 40 sterile plates with 25 mL each

Appendix B

Graduated cylinders

- Labeling: A-0 mM, B-0 mM, C-0 mM, D-0 mM
- No H_2O_2 added (0 mM)

Volumetric flasks

- Labeling: A-2 mM, A-4 mM, B-2 mM, B-4 mM, C-2 mM, C-4 mM, D-2 mM, D-4 mM
- 2.04 μL 30% H_2O_2 added to each flask labeled with 2 mM
- 4.08 μL 30% H_2O_2 added to each flask labeled with 4 mM