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Antioxidant Properties of Melanocytes and their Potential Implication in Neurodegenerative Disorders

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ABSTRACT

Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) and the cellular antioxidant defense system, leads to significant cellular damage. When the body's detoxification mechanisms are overwhelmed, ROS can inflict damage on lipids, proteins, and DNA, resulting in harms in the cells. Antioxidants neutralize these ROS by converting H_2O_2 into water and oxygen, thereby mitigating oxidative damage. Oxidative stress is prominently associated with neurodegenerative diseases. Increased oxidative damage markers have been observed in the brains of patients with neurodegenerative disorders. This study investigates the potential antioxidant properties of melanocytes, pigment-producing cells as a model to understand ROS-induced damage and its relevance to neurological disorders. Using ROS measurement, cell viability assay, and catalase activity tests, this study demonstrated that melanocytes exhibit significant resistance to ROS. Melanocytes maintained higher catalase activity and cell viability compared to T-MSCs under oxidative stress conditions. These findings suggest that melanocytes possess robust mechanisms for mitigating ROS-induced damage, making them a valuable model for exploring oxidative stress-related neurological disorders.

Keywords: Melanocytes, Reactive Oxygen Species, Oxidative Stress, Catalase, Neurodegenerative Disorders

INTRODUCTION

Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and the organisms' antioxidant defense mechanism, leading to cellular damage. ROS, including superoxide radicals(O2-) and hydrogen peroxide (H2O2), are produced as natural byproducts of cellular metabolism, such as mitochondrial respiration, inflammation, and enzymatic reactions involving oxidases. When the human body lacks the ability to detoxify them, ROS can react with various cellular components, such as lipids, proteins, and DNA. This leads to oxidative damage, including lipid peroxidation, damaging the cell membranes, protein oxidation, which can alter protein function, and DNA damage, which can cause lethal genetic mutations. To prevent the ROS from accumulating in cells and tissues, body naturally produces antioxidants, such as enzymes such as glutathione, catalase or vitamins which break down hydrogen peroxide, into water and oxygen, decreasing the free radicals in the body.

Previous research highlights oxidative stress impact on various neurodegenerative diseases. Increased oxidative stress is a common feature in various

neurodegenerative diseases, often linked to aging.[1] Markers of oxidative damage, such as protein nitration, lipid peroxidation, have been observed to be elevated in the brains of neurodegenerative disease patients. Disruptions in the physiologic maintenance of the redox potential in neurons interfere with several biological processes, ultimately leading to neuronal death. It has been suggested that oxidative stress is one of the main causes of Parkinson's disease, inducing the degeneration of dopaminergic neurons.[6] Also, researches have shown excessive hydrogen peroxide originated from severe reactive astrocytes causes tauopathy, neuronal death, brain atrophy, and cognitive impairment, ultimately leading to the development of Alzheimer's disease.[3]

Although there were many attempts to find a successful antioxidant that can cure neurodegenerative diseases, no treatments have shown significant responses till now.[7] In this research, I tackled melanocyte. Melanocytes are pigment-producing cells that originate from the dorsal portions of the closing neural tube in vertebrate embryos. Recent papers have shown melanocytes found in the retina and inner ear, contribute to vision and hearing and may have antioxidant properties.[1] Furthermore, it has been proposed that in the molecular biological perspective, melanocytes share common embryologic origin, signaling molecules, receptors, and signaling pathways with cells of the nervous system, suggesting that melanocytes can be used as appealing model system to study the disorders that affect the nervous system.[2]

However, despite these findings, the role of melanocytes as an antioxidant and its impact of ROS on melanocyte functions have not yet been fully explored. Furthermore, applying its role as an antioxidant in developing potential therapies for oxidative stress induced neurodegenerative diseases has not been investigated.

This study investigates the effects of reactive oxygen species on melanocytes and their potential relevance to neurological disorders. This study aims to understand the underlying mechanism of ROS-induced damage in melanocytes and its contribution to neurological disorders, such as Parkinson's disease and Alzheimer's disease. Using ROS measurement, cell viability test, and catalase activity test, this study demonstrates that melanocytes have a profound resistance to ROS and show significantly less damage when treated with ROS than the control group. Based on these findings, I propose melanocytes as a novel model system in which to study neurological disorders, and that it may facilitate the development of a therapeutic interventions for neurological disorders involving melanocyte dysfunction.

MATERIALS & METHODS

I. Cell Culture (Melanocytes and T-MSC)

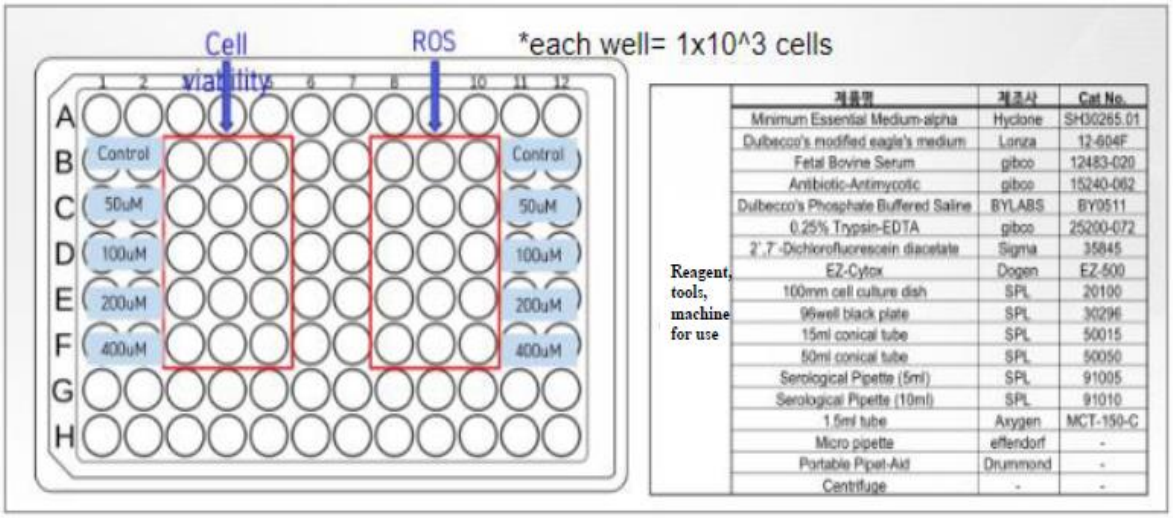
In this research, tonsil derived-mesenchymal stem cells (T-MSC) were used as a control group. First, mouse melanocytes were cultured in a 100mm plate with media composed of Dulbecco's Modified Eagle Medium (DMEM), 10% FBS, 1% penicillin-streptomycin. and it was incubated in a CO2 incubator of 37 degrees celsius. The T-MSC cells were also cultured in a 100mm plate with the same media but with modified eagle medium (MEM) and were incubated in the same environment.

II. ROS Treatment and Measurement

For ROS measurement and cell viability test (CCK), the cells were subcultured in 2 different black wall clear bottom 96 well. One for melanocytes and one for T-MSC. The cells were subcultured in the 15 designated wells as seen in Fig 1, each well set with 1*10^3 cells. After an incubation overnight, both cells were treated with 100ul of 10uM DCFDA in different concentrations, so that each group was treated with 0 H2O2, 50uM, 100, 200, 400uM. Then the cells were incubated for 30 minutes in the dark. After 30 minutes, we measured the fluorescence using a

spectrofluorometer at 488 nm excitation and 525 nm emission.

Fig .1 Cell Viability Test and ROS measurement experiment design



III. Cell Viability Test (CCK)

Simultaneously, cell viability test was performed on melanocytes and T-MSC in the left 15 designated wells. First, different amounts of hydrogen peroxide were added to the cells. Fresh media was added with the H2O2 treatments for 24 hours. and on the next day, CCK reagent together with media was treated. Plates were covered with foil and after 1 to 4 hours of co2 incubation, the absorbance was measured at 450nm in the SpectraMax ABS Plus microplate reader.

IV. Catalase assay (Metabolic Activity Assessment)

To assess the metabolic activity of the cells, the catalase assay was conducted. For this experiment, dogen bio ez-catalase assay kit was used. For this assay, the cells were cultured in two 6 well plates. After harvesting the cells, they were centrifuged and collected the supernatant. The supernatants of each group were added with 40um H2O2 and was loaded in 96 wells. Then it was covered with foil and incubated in room temperature for 30 mins. After another treatment for oxi-probe solution and incubation, the absorbance was measured at 560 nm in the microplate reader.

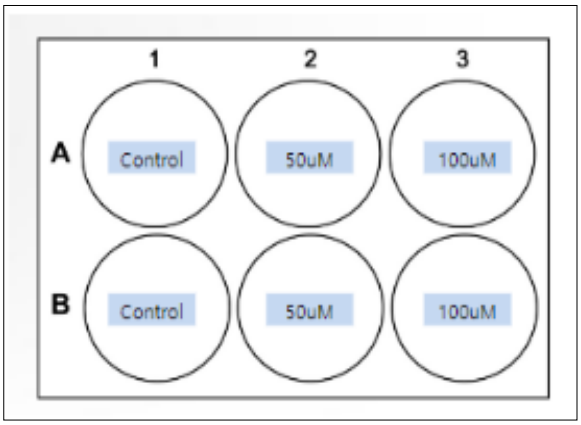


Fig.2 Plate design for catalase assay

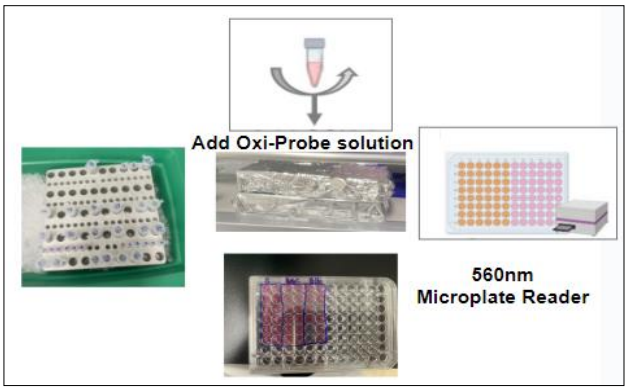


Fig. 3 Catalase assay experiment design

RESULT

I. Cell Culture

Fig.4 a. Microscope image of MSC observed at 10X magnification immediately after cell culture. b Microscope image of MSC observed at 4X magnification immediately after cell culture. c Hemocytometer image of MSC after culture, 7 x 10 ^{4} cells/ml

II. ROS measurement and Cell Viability Test (CCK) Result

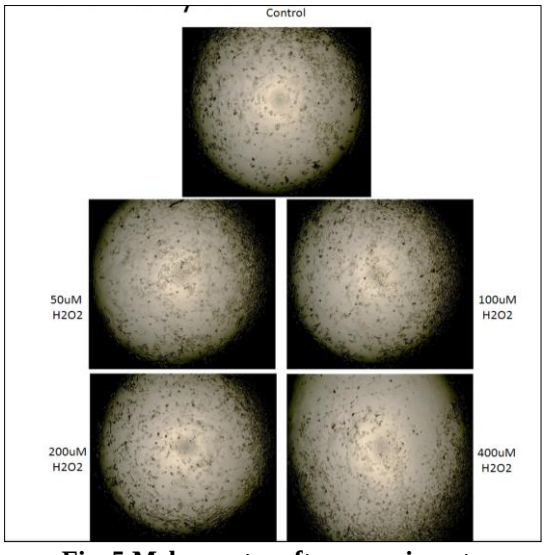


Fig. 5 Melanocytes after experiment

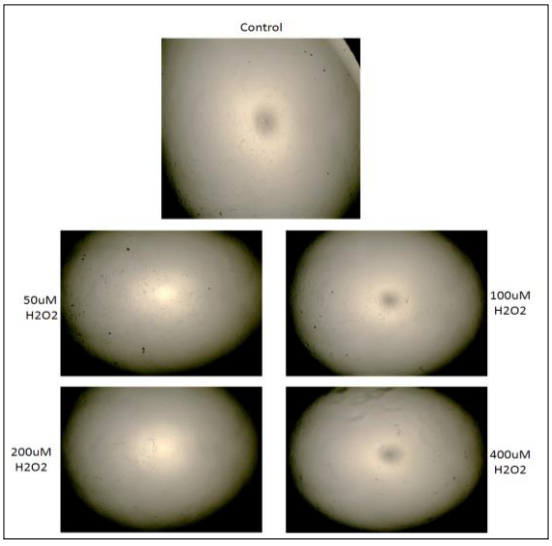


Fig. 6 MSC after experiment

This stereomicroscope image depicts melanocytes and MSCs (mesenchymal stem cells) treated with varying concentrations of H2O2. A noticeable reduction in cell numbers is observed at both the 200 µM and 400 µM H2O2 concentrations. At 200 µM, both MSCs and melanocytes exhibit a decrease in cell count. However, at 400 µM, MSCs are entirely absent, whereas a substantial number of melanocytes remain

viable. These observations suggest that melanocytes are relatively more resistant to oxidative stress induced by ROS (reactive oxygen species) compared to MSCs.

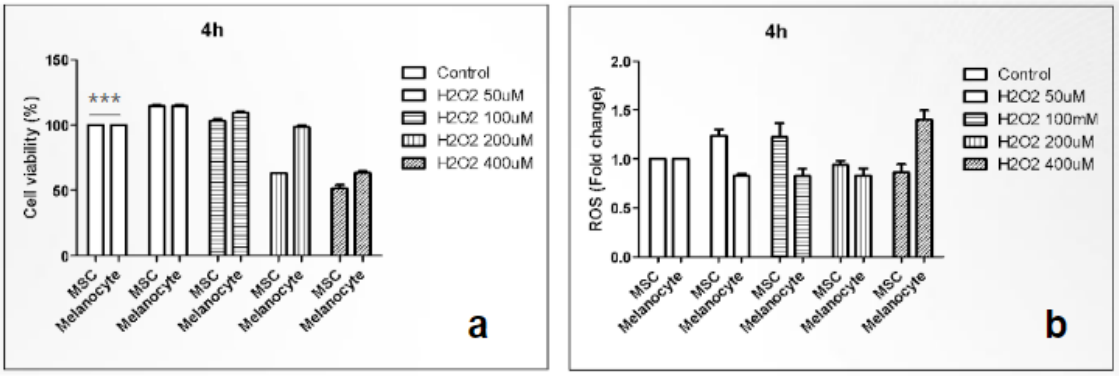


Fig 7 a. Summary bar graph of cell viability of MSC and Melanocyte after 4 hours of H2O2 treatment One way ANOVA, MSC vs Melanocyte, p<0.01 b. Summary bar graph of the fold change of ROS after 4 hours of H2O2 treatment

The following are the results from the cytotoxicity and ROS measurement experiments. As expected based on theoretical principles, Figure 7-a illustrates a decrease in cell viability with increasing H2O2 concentrations. Hydrogen peroxide concentrations above 200 µM adversely affect cellular mechanisms, leading to cell death when the oxidative stress exceeds the cell's capacity to neutralize it. Figure 7-a clearly shows that melanocytes have a significantly higher survival rate compared to MSCs across the 100 µM, 200 µM, and 400 µM H2O2 treatment groups. Notably, in all groups, melanocytes demonstrate either comparable or superior cell viability relative to MSCs.

Figure 7-b presents a bar graph depicting ROS levels in melanocytes and MSCs across different treatment groups. This graph quantifies the intracellular production of free radicals, with higher ROS values indicating greater oxidative stress and increased cell death. Conversely, lower ROS levels suggest enhanced activity of intracellular antioxidant enzymes, which reduce free radicals by decomposing hydrogen peroxide. In all groups except the 400 µM group, melanocytes showed a reduction in ROS levels at 50, 100, and 200 µM, indicating effective metabolic degradation of hydrogen peroxide. The unexpectedly high ROS value observed in the 400 µM group for melanocytes could be attributed to measurement or calculation errors, or it may indicate that at this concentration, melanocytes are less efficient at decomposing reactive oxygen species compared to lower H2O2 concentrations.

III. Catalse Assay Result

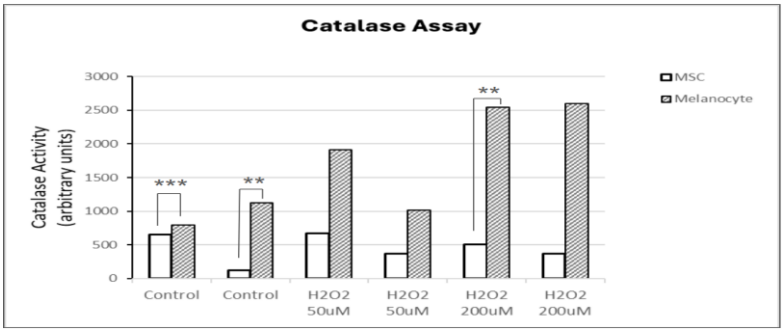


Fig 8 Bar graph of catalase activity of MSC and melanocyte treated with 0µM, 50µM, 200µM of hydrogen peroxide. Unpaired t-test, p<0.001; Control MSC vs Control Melanocyte, One-way ANOVA, p<0.05; 200µM MSC vs 200µM Melanocyte

Catalase activity in melanocytes is consistently higher than in MSCs across all experimental groups. Notably, in the 200 μM H2O2 group, catalase activity in melanocytes is approximately six times greater than in MSCs. This finding suggests that melanocytes exhibit a significantly higher resistance to ROS, as they maintain elevated levels of active catalase within the cells, which continues to degrade ROS. The congruence of results from the cytotoxicity assays, ROS measurements, and catalase activity data confirms that melanocytes sustain less damage from ROS and demonstrate greater resistance compared to MSCs.

DISCUSSION

The results of the three experiments collectively support a consistent conclusion: melanocytes exhibit greater resistance to hydrogen peroxide compared to MSCs. Data on catalase activity, ROS levels, and cell viability demonstrate that melanocytes maintain higher catalase activity, which continues to degrade ROS, leading to increased cell survival. Melanocyte is remained as a hidden cell in the nervous system. Thus, not many studies have tackled melanocytes. However, it is still considered to be pluripotent.

To further elucidate the mechanisms underlying the heightened ROS tolerance observed in melanocytes, future studies are planned to analyze the expression levels of key genes associated with melanocyte function using qRT-PCR. This analysis will allow for a comparison of gene expression profiles between control and ROS-treated groups. Additionally, further research aims to investigate the relationship between changes in dopaminergic neurons and melanin production by comparing the levels of neuromelanin in post-mortem brain tissues from Parkinson's disease patients.

This study provides a realistic perspective on the impact of ROS on melanocyte function and its potential link to neurological diseases. By enhancing our understanding of the mechanisms of ROS-induced damage in melanocytes, this research could contribute to the development of improved therapeutic strategies for neurological disorders. By harnessing the potential of melanocytes, this work seeks to pave the way for the development of effective treatments, ultimately advancing toward a future where neurological disorders no longer impose suffering on patients and future generations.

CONCLUSION

In conclusion, this study demonstrated that melanocytes have recognizably high antioxidant abilities compared to mesenchymal stem cells. By measuring the reactive oxygen species expressed from the melanocytes due to external stimulation compared to different cells, this paper was able to show that melanocytes have high resistance to environment besides mesenchymal stem cells. This study has unlocked the potential of melanocytes by highlighting the outstanding antioxidant properties of it. Furthermore, it has proposed ways to new treatments of neurological disorders via stimulation of melanocytes, giving hope to patients genuinely awaiting for a successful therapy.

Declaration by Authors

Ethical Approval: Approved

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Conflict of Interest: The authors declare no conflict of interest.

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