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CBD and Alzheimer’s Disease; Neuroprotection and Desensitization

Introduction

Alzheimer’s Disease (AD) is a neurodegenerative disease ranked as the sixth leading cause of death in the United States. Additionally, it is the leading cause of dementia in adults [1]. Alzheimer’s Disease is primarily characterized by the presence of β-Amyloid plaques (Aβ) and Neurofibrillary tangles (NFTs). Aβ plaques are extracellular, toxic, misfolded proteins that clump together between neurons causing the obstruction of cell signaling, damaging neurons, and eventually leading to cell death via apoptosis [2]. Studies have shown that oxidative stress also plays a major role in the pathogenesis of AD in relation to the presence of Aβ. Specifically, research has shown that Oxidative stress is highly associated with Aβ plaque formation and the consequent neuronal degeneration, facilitating progression of AD and cell death via apoptosis [3].

Cannabidiol (CBD) is a non-psychotropic phytocannabinoid extracted from Cannabis sativa. CBD recently attracted research interest due to its antidepressant, anticonvulsive, antioxidant, and neuroprotective effects [4]. Due to its neuroprotective properties against Aβ toxicity, CBD is showing promise as a treatment for AD. In recent studies, CBD has been linked to exerting its neuroprotective effects on neuronal cells [5,6]. However, the mechanisms underlying this phenomenon are not fully understood. In addition, none of those studies considered desensitization and time-dependent response. Addressing the time-dependent response of the neuroprotective properties of CBD provides important information that can help optimize the use of the drug in clinical settings.

Our goal in this research was to investigate the neuroprotective effect of CBD on SH-SY5Y cells, a human neuroblastoma cell model of human neurodegenerative disease, in the presence of Aβ peptides. Additionally, we explored whether CBD neuroprotective properties will be abolished by desensitization
over long-term exposure. **Our hypothesis** for this research was that CBD can increase cell viability and decrease oxidative stress; however, the effect will decrease in a time-dependent response.

**Methods:**

*Cell Culturing:*

For this experiment we utilized SH-SY5Y cell line. This cell line can be used in different types of neurological studies including those related to neurodegenerative processes, neurotoxicity, and neuroprotection [7]. The cells were thawed and placed in a T-25 along with growth medium. The cells were then incubated at 37°C (body temperature), 5% CO₂ to help maintain the pH of the cell culture. Cells were monitored for confluence, and growth medium was refreshed as needed.

The cells were then moved from the T-25 flask to a 96-well plate where they will undergo treatment. In order to move the cells, the growth medium was first removed from the T-25 flask and 1mL of trypsin was added. The T-25 flask was lightly tapped for 6 minutes so that the cells would lift from the surface of the T-25 flask. Then 3mL of growth media were added to neutralize the trypsin. Then, 1mL of cell suspension was added to a centrifuge tube (total of 4 centrifuge tubes). The centrifuge tubes were centrifuged at 1400rpm for 5 minutes. The solution was then removed from the centrifuge tubes, leaving behind just the pellet of cells. The pellet of cells was then resuspended by adding 800 μl of fresh media and aspirating. This cell solution was then transferred into a conical tube and 10 μl of cell solution was obtained and loaded into a hemocytometer for counting. After counting we plated 100,000 cells/well on the 96 well plates.

Once the cells were plated into the 96-well plate they were incubated at 37°C, and 5% CO₂ for 24 hours to allow the cells to attach to the bottom of the plate. After the 24 hours the growth media was removed, and FBS-free media was plated, and the cells were incubated under the same conditions as before for another 24 hours. At this point, cells were ready to be treated with CBD and Aβ peptides.

*MTT assay:*

MTT assay was split into 3 days, on the first day we treated the cells with Aβ-42 oligomer and CBD. Wells A2, A3, A4, A6, A7, and A8 were treated with 100 μl of CBD 30 minutes prior to the treatment with Aβ-42 oligomer. After 30 minutes columns 6, 7, and 8 were all treated with 4 μl of Aβ-42 oligomer.
Row B was immediately treated with 100 μl CBD. Then Rows C-H were treated with the same 100 μl CBD consecutively every 30 minutes (Figure 1). After the treatments were done the cells were incubated under the same conditions as before for another 24 hours. On day two the actual assay was performed. First, 5 mL of 1mg/mL MTT was prepared by diluting 1mL of 5mg/mL of MTT and 4 mL of FBS free media. Then an SDS solution was made to be used later to dissolve the crystals made by the MTT solution. This was made by mixing 1g SDS and 100 μl of 1N HCl and water up to 10mL. The treatment solutions were then removed from each well, and 50 μl of 1mg/mL MTT was added to each well and incubated for 3 hours under the same conditions as before. After the 3 hours, 100 μl of the SDS solution was added to each well and incubated under the same conditions for 18 hours, so the solubilization solution could dissolve the crystals that were made by MTT solution. After the 18 hours the plate was read by the synergy H1 microplate reader and results were obtained.

**ROS assay:**

For the ROS assay first, all reagents were prepared following the instructions from the kit. The ROS reagent was dissolved in 60 μl DMF to make a 5mM stock. The ROS inducer which was used as a positive control was dissolved in 100 μl DMF to make 10mM stock. The ROS inhibitor which was used as the negative control was dissolved in 123 μl DI water to produce 500mM stock. Then ROS solution was prepared by adding 5mL of 1x wash buffer to 1 μl of the prepared ROS reagent. Once these were prepared, the supernatant was removed from the 96 well plate and the cells were washed with 1x wash buffer. Then 100 μl of ROS solution was added to each well. Additionally, 11.2 μl of positive control (ROS inducer) and 1 μl of
negative control (ROS inhibitor) were added to wells designated as control. The cells were incubated with the ROS treatment under the same conditions previously mentioned for 1 hour. After one hour the cells were treated as shown in Figure 2 with 4 μl of Aβ-42 oligomer, and 100 μl CBD.

The plate was immediately put in the synergy H1 microplate reader and results were obtained.

**Results and discussion:**

MTT assay results shows that CBD is neuroprotective against Aβ oligomer excitotoxicity (Figure 3).

ROS assay indicates that both CBD and Aβ oligomer increase the ROS in the SH-SY5Y cells (Figure 4), however, ROS production decrease over time (Figure 5).

![MTT assay](image)

Data represent the average and standard error of three independent experiments (each triplicate). * p < 0.05 drug treatment vs. vehicle ANOVA analysis with Tukey’s post-test.

![ROS Assay](image)

Data represent the average and standard error of two independent experiments (each triplicate). * p < 0.05 drug treatment vs. vehicle ANOVA analysis with Tukey’s post-test.
A part of my results has been presented at the Texas Society of Genetics (TSG) in 2022.

**Goals:**

When I attended my first orientation as a newly accepted freshman at Lamar University, I remember Dr. Terry telling my group about undergraduate research. Although, I found it very interesting, I never thought I would actually have the opportunity to be a part of it. This O.U.R grant allowed me the opportunity to gain experience working in lab. I began training at the end of the spring 2021 semester with training in basic cell culturing techniques such as feeding cells and splitting cells. I also completed all my CITI trainings and Dr. Vasefi ordered all the materials we were going to need for the project.

One challenge that we had to overcome was working around delays of our materials that we ordered. Although, this was a setback it helped me witness some of the things that can go wrong when working in a lab setting and how one can work around those setbacks. Dr. Vasefi was able to borrow the materials we were missing and this way we were able to get started with the project and conduct MTT and ROS assays.

Working on this project with Dr. Vasefi introduced me to a new professional setting that I wouldn’t have ever experienced if I hadn’t received the O.U.R support. Although, my plan is to go to medical school and eventually become a doctor, the time I spent in the lab with Dr. Vasefi and graduate students (Anthony Osu, Hy Lai, and Claire Alexander) really fostered a new interest in me and motivated me to continue to pursue research for my remaining time at Lamar.

In addition, I will continue the research project in Dr. Vasefi’s lab in Summer 2022.

**Bibliography:**


