12-6-2022

Impact of Cannabinoids on Human Neural Differentiation and Oxidative Stress Response

Emily Brothersen
Utah State University, emily.brothersen@usu.edu

Dillon Weatherston
Utah State University, dillon.weatherston@usu.edu

Ashton Young
Utah State University, A00302384@usu.edu

Bryan Gustafson
Utah State University, bryancg32@gmail.com

Impact of cannabinoids on human neural differentiation and oxidative stress response

Emily Brothersen, Bryan Gustafson, Dillon Weatherston, Ashton Young
Utah State University

INTRODUCTION

Cannabinoids are a group of over 144 known compounds extracted from hemp species. (1) Tetrahydrocannabinol (THC), the psychoactive compound in hemp, and the non-psychoactive cannabidiol (CBD) are the most commonly known and most well studied cannabinoids. As cannabinoids can be sold for medicinal or recreational purposes (2), it's important to understand the impact of these cannabinoids on the brain.

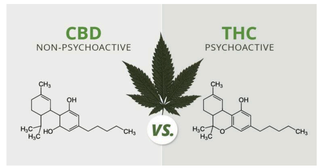

Figure 1: Molecular structure of CBD vs THC (3)

One vulnerable population whose interaction with cannabinoids has not been thoroughly studied is pregnant women and developing fetuses.

To explore the effects of cannabinoids on neural development, undifferentiated SH-SY5Y human neuroblastoma cells will be used as a model of fetal neurons:

- SH-SY5Y cells were fully differentiated in the presence of no cannabinoids, THC, and/or CBD and analyzed for any developmental differences
- As cannabinoids have previously demonstrated protective antioxidant properties, the cytoprotective properties of THC and CBD on differentiated and undifferentiated neurons were also explored

METHODOLOGY

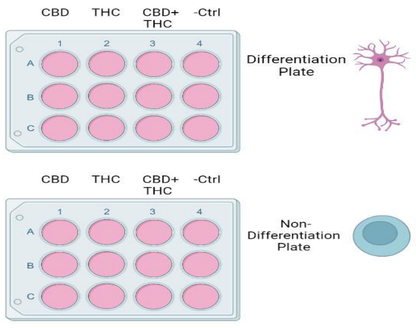

Figure 2: Depiction of the experimental layout for differentiation

- Human neural line SH-SY5Y was cultured for 4 days in 12-well plates
- On day 4, cannabinoid treatments were added and Phase I of differentiation began (4)
- 3 days later, phase II of differentiation began and treatments were replaced (4)
- After 3 more days of differentiation, hydrogen peroxide (5) was added to some groups to create Reactive Oxygen Species (ROS)
- On day 11, viability stains and quantification of ROS were performed

RESULTS

Neuron Differentiation:

- Qualitative analysis of neural differentiation was determined by dendrite formation at day 7
- As shown in **Figure 3**, there is no significant difference in neural differentiation after 7 days following treatment of CBD, THC, or a combination of both chemicals

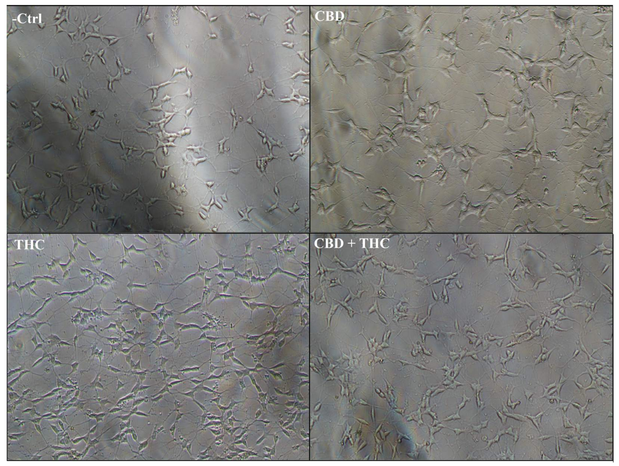

Figure 3: Micrograph of each group taken 7 days post-differentiation and treatment. Taken at 4x magnification

Protection from Oxidative Stress:

- There was no significant effect on ROS from the differentiation protocol and treatment groups
- There were no interaction effects between the treatment groups and the differentiation protocol
- There is a significant interaction effect between H2O2 and the treatment groups. In other words: at least one of the treatment groups significantly changes the amount of ROS in the presence of H2O2

Table 1: 3 way ANOVA was performed with type 3 Sum of Squares to determine which variables effected the amount of ROS

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Differentiation	1	3.46150	3.46150	0.47	0.4965
Treatment	3	7.47447	2.49149	0.34	0.7967
H2O2	1	69072.80672	69072.80672	9418.46	<.0001
Differenti*Treatment	3	8.97579	2.99193	0.41	0.7482
Treatment*H2O2	3	136.61171	45.53724	6.21	0.0016

To determine how each treatment affects the ROS response, the data were plotted in Figure 3, which shows no significant difference in ROS among all groups that did not receive the oxidative stress treatment, and slightly significant improvement against oxidative stress in the presence of CBD, but not in the presence of THC or a combination of CBD and THC.

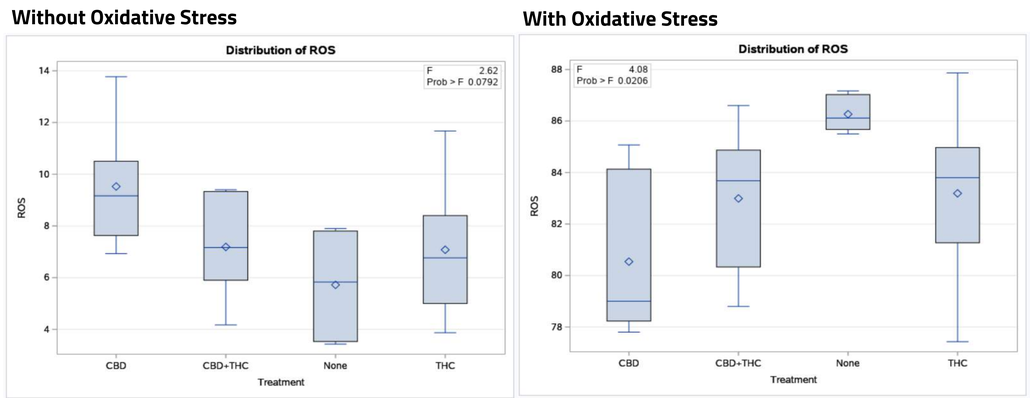

Figure 4: Distribution of ROS in response to each treatment. There is no significant difference in the amount of ROS in the absence of H2O2. In the presence of H2O2, there is a significant but slight reduction in the amount of ROS when CBD is added.

Cell Viability Analysis:

- Cell viability was determined qualitatively through Calcein AM, and EthD-III stain
- No significant difference in cell viability was detected among any of the treatment groups before or after differentiation
- Cell growth was comparable among all treatment groups

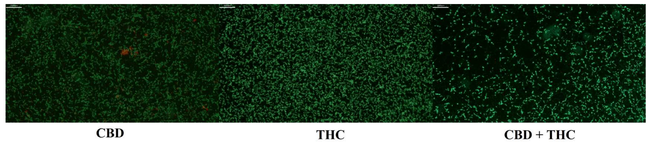

Figure 5: Live-Dead stain of differentiated SH-SY5Y cells at day 7. Calcein AM(green) stained cells indicate living cells. EthD-III(red) cells indicate dead cells. None of the treatment groups showed significant cell death.

CONCLUSION

This experiment aimed to determine if cannabinoids had any effect on neuron development. Using dendrite formation of human neural cell line SH-SY5Y, we were unable to show any impact of the cannabinoids THC and CBD on neuron differentiation. We also measured the protective effects of these cannabinoids against ROS and determined that CBD provides a mild protective effect against oxidative stress.
Future experiments can be performed to optimize the concentration at which CBD provides a protective effect against ROS as well as determining if there is a concentration at which THC can provide significant effects against ROS.

REFERENCES

1.Hanuš, L. O., Meyer, S. M., Muñoz, E., Taglialatela-Scafati, O. & Appendino, G. Phytocannabinoids: a unified critical inventory. *Nat. Prod. Rep.* **33**, 1357–1392 (2016).
2.Giacoppo, S., Mandolino, G., Galuppo, M., Bramanti, P. & Mazzon, E. Cannabinoids: New Promising Agents in the Treatment of Neurological Diseases. *Molecules* **19**, 18781–18816 (2014).
3.The Lighthouse Marijuana Dispensary Near Me | Palm Springs, Coachella. *Medical Marijuana The Lighthouse Dispensary* https://www.lighthousedispensary.com/.
4.Forster, J. I. *et al.* Characterization of Differentiated SH-SY5Y as Neuronal Screening Model Reveals Increased Oxidative Vulnerability. *J. Biomol. Screen.* **21**, 496–509 (2016).
5.Zhang, L. *et al.* Protective effects of salidroside on hydrogen peroxide-induced apoptosis in SH-SY5Y human neuroblastoma cells. *Eur. J. Pharmacol.* **564**, 18–25 (2007).

ACKNOWLEDGEMENTS

We would like to acknowledge Dr. Elizabeth Vargis for allowing us to use her lab, equipment, and supplies Dr. Anhong Zhou for SH-SY5Y cells, and Emilee Rickenbaugh for tissue culture support.