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Effects of Cannabidiol on *Dugesia Dorotocephala* Head Regeneration

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The endocannabinoid system regulates synaptic transmissions. It is comprised of two G protein-coupled receptors, cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2), a degradation system and the endocannabinoids, a group of lipidic ligands. The connection between JNK, cannabinoids, and regeneration has led to the hypothesis that cannabinoids impact both regeneration and the levels of the enzymes required for the regenerative process. Thus, by encouraging neoblasts to enter the M phase, cannabinoids would speed up effective regeneration. These regeneration pathways may have implications in cancer research. In addition, CB1 reception suppresses the growth of hepatocellular carcinoma, indicating that the endocannabinoid system may be a possible course of treatment for cancer patients. CB2 receptors in nonimmune cells have revealed the benefits of agonists on osteoporosis and post-ischemic heart failure. In order to understand the effects of cannabidiol on the regenerative process and neural transmission, we conducted experiments on Dugesia dorotocephala. Dugesia dorotocephala is an ideal candidate for the endocannabinoid model because they are more genetically uniform than most natural populations and their regeneration is specifically tied to cannabinoid receptors. We transversally cut twenty-four Dugesia dorotocephala to analyze differences in head regeneration in solutions with varying amounts of cannabidiol. We found that Dugesia dorotocephala in CBD solutions have a faster rate of head regeneration, yet our results were not statistically significant. The increased rate of head regeneration in Dugesia dorotocephala in CBD solution may be attributed to the stimulation of neoblasts to enter the M-Phase of the cell cycle.

Keywords: *cannabidiol (CBD), planaria, regeneration, endocannabinoid*

Introduction

The endocannabinoid system regulates synaptic transmissions. It is comprised of two G protein-coupled receptors, cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2), as well as a degradation system and the endocannabinoids, a group of lipidic ligands (Mallat and Lotersztajn 2008). Cannabinoid receptors are primarily located on presynaptic neurons, where they inhibit neurotransmitter release (Buttarelli et al. 2004). While CB1 is primarily located in the central nervous system and peripheral tissues, CB2 receptors are primarily located in immune cells (Pacher et al. 2006).

In order to understand the effects of cannabidiol on the regenerative process and neural transmission, we conducted experiments on *Dugesia dorotocephala*. *Dugesia dorotocephala* is an ideal candidate for the endocannabinoid model because they are more genetically uniform than most natural populations (Pagan 2017). In addition, Planaria are becoming increasingly popular models in developmental and regenerative pharmacology. This is likely due to their relatively

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complex organ structure, including their ability to, in some cases, completely regenerate their brain and nervous system (Roberts-Galbraith and Brubacher 2016).

Planaria have shown a dose-dependent increase in movement when exposed to the cannabinoid WIN55212-2 (Buttarelli et al. 2002). After removal of WIN55212-2, planaria display withdrawal symptoms of decreased motor function (Rawls et al. 2007). Planarian regeneration is specifically tied to cannabinoid receptors. In 2000, the connection between c-Jun N-terminal kinase (JNK) and the cannabinoid receptor, CB_1, was discovered. By transfecting Chinese hamster ovary cells with CB(1) receptor cDNA, the study showed that that Delta(9)-tetrahydrocannabinol (THC), endogenous cannabinoids (anandamide and 2-arachidonoylglycerol), and synthetic cannabinoids (CP-55,940, HU-210, and methanandamide), induce the activation of c-Jun N-terminal kinase (JNK) (Rueda et al. 2000). In 2011, it was discovered that JNK inhibition prohibits regeneration because it prevents neoblasts from progressing into the M-Phase of the cell cycle (Tasaki et al. 2011). The connection between JNK, cannabinoids, and regeneration has led to the hypothesis that cannabinoids impact both regeneration and the levels of the enzymes required for the regenerative process (Blasiman 2013). Thus, cannabinoids should lead to M-Phase progression. Neoblasts, proliferating cells that produce undifferentiated cells, underlie the regenerative process of planarians (Wagner et al. 2011). Without the mitotic phase of the cell cycle, the neoblasts cannot reproduce and undergo regeneration. Thus, by encouraging neoblasts to enter the M phase, cannabinoids would speed up effective regeneration. In contrast, researchers have also found that CBD concentrations greater than 2µM inhibit cell activity and growth in a dose-dependent manner; this value rises to concentrations greater than 10µM in oral cell populations (Pagano et al. 2020). Furthermore, cell migration and proliferation were found to be significantly inhibited by CBD doses.

In contrast, some studies point to cannabidiol as an anxiolytic. Anxiolytics target messengers in the brain and decrease excitability. A literature review of pre-clinical and clinical studies found an association between synthetic cannabinoids and executive function impairment (Cohen and Weinsten 2018). In addition, CBD has been found to disrupt interactions between the amygdala and anterior cingulate cortex, via the reduction of blood oxygenation level-dependent signals in the amygdala, which resulted in the suppression of both amygdalar and anterior cingulate responses (Fusar-Poli et al. 2010). In clinical terms, the anxiolytic effects of CBD lead to its use as an anxiety drug, but the mechanisms and proper dosage of CBD for this purpose is still undetermined (Resstel et al. 2009).

Our goal was to answer the question, "How does cannabidiol (CBD) affect rates of head regeneration in *Dugesia Dorotocephala*?" We hypothesized that CBD would slow down the developmental processes of the *Dugesia Dorotocephala*, resulting in a longer period of regeneration. We predicted that if CBD slowed the developmental processes of *Dugesia Dorotocephala*, it would take more time for *Dugesia Dorotocephala* in CBD solution to regenerate their heads than *Dugesia Dorotocephala* in water solution.

Materials and Methods

The experiment involved transversally cutting twenty-four *Dugesia Dorotocephala*. The *Dugesia Dorotocephala* were divided into four groups, so there were five replicates of each solution. Each group resided in a different solution (see Table 1).

Table 1. *Dugesia Dorotocephala Grouping into Various Solutions*

Solution	Solution Contents	Planaria #s	Solution pH
A	100 % Bliss Water (CBD water)	1-6	6
B	50% Bliss Water, 50% Spring Water	7-12	6.5
C	25% Bliss Water, 75% Spring Water	13-18	7
D	100% Spring Water	19-24	7

Solutions were made in 100 mL batches. After each solution was pH tested, 5 mL of CBD solution was placed into each well for the corresponding *Dugesia Dorotocephala*. The *Dugesia Dorotocephala* were transected individually on a Kimwipe-covered ice cube in a petri dish under a dissecting microscope and then were placed into their corresponding wells. The *Dugesia Dorotocephala* resided in a dark cabinet at room temperature following transection.

Dugesia Dorotocephala regeneration was monitored under a dissecting microscope every one to two days throughout the experiment. Regeneration was based on the scale below (Table 2).

Table 2. *Dugesia Dorotocephala Regeneration Criteria*

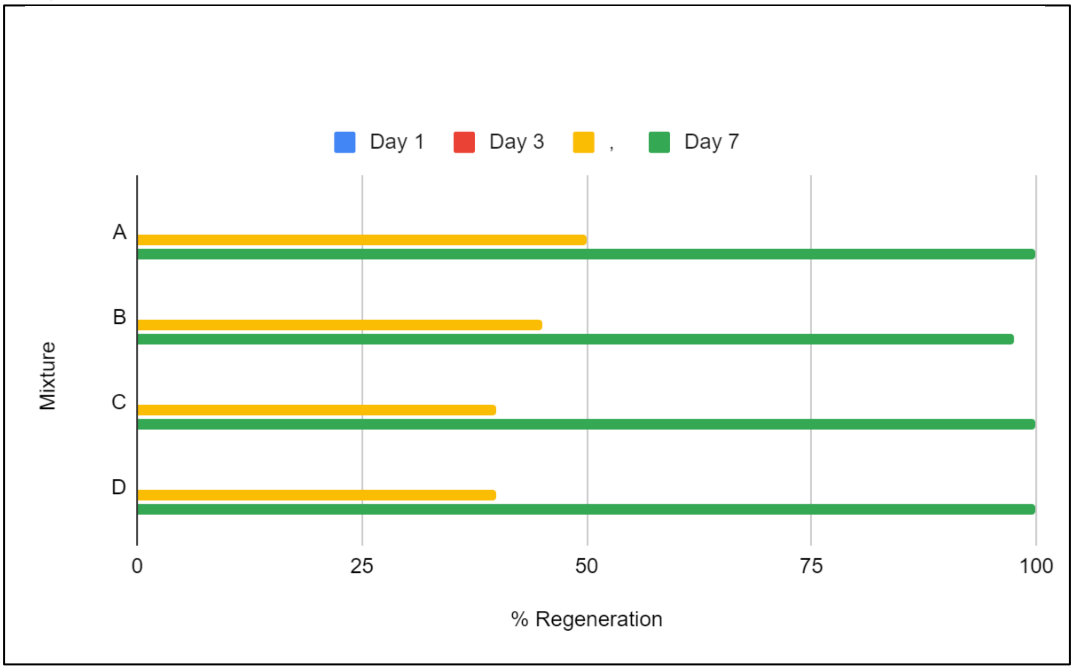
Percent Regeneration (%)	Criteria
0	Just tail
45	Rounded top
50	Pointy top
75	One eye
100	Both eyes

Results

Research was conducted to determine the effects of cannabidiol on head regeneration. The mean rates of head regeneration for each solution are graphed in Figure 1, revealing that solutions with more cannabidiol produced faster rates of

Dugesia Dorotocephala head regeneration. The series correspond to the days that the planaria growth was reported. Planaria growth was reported every two days.

Figure 1. *Comparison on Rates of Planarian Head Regeneration in Solutions with Varying Amounts of Cannabidiol*



Dugesia Dorotocephala in solutions with more cannabidiol had faster blastema formation. While there was a difference in the rates of regeneration, the difference was slight and may have been due to other factors. The results of the P-Test reveal that the difference in regeneration is not statistically significant (Table 3).

Table 3. *Comparison of 100% Bliss Water and 100% Spring Water*

Solution	**Percent Regeneration (%) Day One**	**Percent Regeneration (%) Day Three**	**Percent Regeneration (%) Day Five**	**Percent Regeneration (%) Day Seven**
100% Bliss Water	0	0	50	100
100% Spring Water	0	0	40	100
P-Value : 0.9432				

Discussion

The experiment was conducted to determine how cannabidiol (CBD) affect rates of head regeneration in *Dugesia Dorotocephala*. Since cannabidiol has been shown to prevent effective interactions between the amygdala and anterior cingulate cortex, resulting in less neural excitability (Fusar-Poli et al. 2010), we expected the *Dugesia Dorotocephala* in solutions with CBD to regenerate their heads slower than those in spring water solutions. As a result, we hypothesized that CBD would slow down the developmental processes of the *Dugesia Dorotocephala*, resulting in a longer period of regeneration and predicted that if CBD slowed the developmental processes of *Dugesia Dorotocephala*, it would take more time for *Dugesia Dorotocephala* in CBD solution to regenerate their heads than *Dugesia Dorotocephala* in water solution. Our findings neither confirmed nor denied our hypothesis. While we found that *Dugesia Dorotocephala* in CBD had faster rates of head regeneration than those in spring water, our data was not statistically significant.

While significant strides in endocannabinoid systems have not been made in Planaria, results have been found in zebrafish. Crude cannabidiol extracts have been shown to reduce apoptosis and promote fin regeneration following zebrafish amputation (Xu et al. 2021). Apoptotic cell death was averted through the ability of CBD to downregulate IL-1β, Caspase 3, and PARP protein expression. Furthermore, the researchers believed that regeneration was enhanced through modulation of host inflammatory response. Our results were consistent with those found in this study, as our data, while not significant, found that CBD promoted regeneration. Therefore, planaria head regeneration may also be mediated through an anti-inflammatory mechanism. A recent study on planaria head regeneration at Harvard Medical School found TRAF3 activation through a Cysteinyl-specialized proresolving mediators (Cys-SPM) pathway. TRAF3 plays a regulating role in mammalian phagocyte functions. Therefore, the presence of this mechanism links the pathway of regeneration with the resolution of infectious inflammation (Chiang et al. 2021). Human studies involving single CBD supplementation in a placebo-controlled crossover study on resistance training and muscle damage found small but significant effects, indicating that CBD may aid in muscle regeneration for athletes that undergo high resistance training (Isenmann et al. 2021). The mechanisms of CBD-regulated regeneration may have similar mechanisms across species.

By interacting with hepatic CB1 receptors, endocannabinoids have shown to induce cell cycle proteins, thus promoting hepatocyte proliferation in the liver. Hepatocellular carcinoma up-regulates the endocannabinoid system, specifically the CB1 receptor system, inducing tumor-promoting genes, such as indoleamine 2, 3-dioxygenase (Mukhopadhyay et al. 2015). In addition, CB1 reception suppresses the growth of hepatocellular carcinoma, indicating that the endocannabinoid system may be a possible course of treatment for cancer patients. Researchers have used RNA sequencing to identify that CBD correlates with decreased expression of cell division, proliferation, and DNA repair genes, resulting in a CBD-mediated cytotoxicity toward head and neck squamous cell carcinoma cells (Go et al. 2020).

In addition, CB2 receptors in nonimmune cells have revealed the benefits of agonists on osteoporosis (Ofek et al. 2006) and post-ischemic heart failure (Defer et al. 2009).

Conclusion

While our results were not statistically significant, the data points did reveal a slight increase in the rate of *Dugesia dorotocephala* head regeneration. The brand of CBD-enhanced water used in the experiment, Bliss Water, contains 8mg of pure cannabidiol in a 700mL bottle for a concentration of approximately 0.01mg/mL. This experiment could be repeated with more highly-concentrated solutions to test for the possibility that *Dugesia dorotocephala* regeneration rate is affected by cannabidiol, but only at high concentrations. The increase in regeneration may be due to connections between JNK and CBD, resulting in the encouragement of neoblasts to enter the M-Phase of the cell cycle. In order to achieve statistically significant results, researchers can try to increase CBD concentrations. Implications of CBD research with respect to regeneration include, but are not limited to, treatment plans for liver degeneration, cancer, osteoporosis, muscle regeneration, and heart failure.

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