The Positive Effects of Medical Cannabinoids on the Dysregulated Endocannabinoid System in Autism Spectrum Disorder Patients

Senior Thesis

April 17, 2023

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ABSTRACT

Autism Spectrum Disorder (ASD) is a developmental disability. The symptoms include a lack of social skills, a need for repetitive patterns of life, difficulty in communication, speaking deficits and numerous other symptoms. While the specific cause is unknown, there is much research being done on mechanisms including dysregulations of the immune system, endocannabinoid system, and overall brain (Nez Govorova 2021, Pol 2021). The Endocannabinoid system is a recently discovered branch of the nervous system composed of endocannabinoids which are produced naturally in the body; lipid-based neurotransmitters that bind to cannabinoid receptors (CBRs) and cannabinoid receptor proteins that are expressed throughout the central nervous system but also found in the lungs, liver, kidneys, and immune system. It regulates homeostasis of the central and peripheral mechanisms of food intake, lipids synthesis and metabolism in muscle cells (Alexandre 2019, Dasran 2022, Su 2021, Finn 2102). Endogenous Cannabinoids are chemical compounds that are produced by the body and act on cannabinoid receptors and alter neurotransmitter release and/or activity in the endocannabinoid system in the brain. Exogenous Cannabinoids such as THC and CBD are extracted from the cannabis plant. CBD has a relaxing effect and THC has a psychoactive, euphoric effect. It has been found that patients with Autism have a lower CB1 receptor expression which releases neurotransmitters as their brain is not releasing enough endogenous cannabinoids to bind to CB1 receptors and results in a lack of homeostasis. This thesis will explore the Endocannabinoid system and target the receptors using mixtures of cannabinoids to alleviate symptoms and improve the overall life for Autistic Spectrum Disorder Patients. Current investigations explore multiple mouse models and use different cannabinoids to treat ASD-like symptoms including CBDV, CBD and THC. Using CBDV in VPA mouse models was found to reduce the symptoms of ASD including excessive grooming time and increased sociability (Zamberletti 2019). These help to decrease repetitive behavior and increase social interactions which were dysregulated in ASD models. Mouse studies have shown that the benefits of a tincture of CBD and THC with a low ratio of THC are significant, including regulating CB1 receptors, eliminating symptoms of ASD for ASD patients (Poleg 2021). In human studies there was a total alleviation of epilepsy for ASD patients with use of CBD:THC mixture and a decrease of at least one or more symptoms in each patient (Fleury-Teixeria, 2019).

OVERVIEW

Autism Spectrum Disorder (ASD) is a disability found at high ratios in the world. It is found in 1 in every 100 children at a 3:1 ratio of men to women (Pol 2021). This disability accounts for a lack of social skills, a need for repetitive patterns of life, difficulty in communication, speaking deficits and numerous other symptoms. The disability is hard to diagnose because it differs from person to person with everyone having different symptoms at diverse levels of severity. The current medication for ASD is an antipsychotic which causes symptoms to mirror depression in patients and helps to minimize symptoms but not get rid of them completely. Therefore, the aim in current research is to eliminate the symptoms by addressing the cause of the disorder and the symptoms. Research has discovered the Endocannabinoid System (ECS) in the brain that is made up of enzymes, neurons, neurotransmitters, and receptors. It works to regulate homeostasis in appetite, social skills, and regulates seizures. In the normal ECS, Anandamide (AEA) and 2-Arachidonoylglycerol (2-Ag) are endogenous cannabinoids found in the body and they combine with the CB1 receptor to express neurotransmitters to have different reactions in the body that regulate homeostasis. In ASD patients, there is a decrease of endocannabinoids AEA and 2-AG leading to seizures, repetitive behavior, and deficits in social skills. Through exogenous cannabinoids like Cannabidiol (CBD) and Tetrahydrocannabinol (THC) to replace decreased endocannabinoids, where the receptor binding returns to normal levels and reverses some of the major ASD symptoms. Grown cannabinoids are concentrations from marijuana plants that are specifically categorized by their biomolecules. The most well-known Cannabinoids include Cannabidiol (CBD) and Tetrahydrocannabinol (THC). CBD is an antipsychotic that is used for anti-anxiety, neuroprotective, pain relief and anti-inflammation (Agarwal 2019, Alexandre 2019, Aran 2021,

Burggren 2019, Finn 2021, Nez Govorova 2021). Antipsychotics reduce delusions, paranoia, anxiety, and agitation. CBD can alleviate pain by reducing inflammation endocannabinoid receptor activity and interacting with neurotransmitters. THC is a psychoactive agent and is used for euphoria, anxiety, pain relief, relaxation, and sleep (Agarwal 2019, Alexandre 2019, Aran 2021, Burggren 2019, Finn 2021, Nez Govorova 2021). Studies have looked at several types of mice to find the most like ASD pathogenesis in behavior. Through these studies, mice are used to mimic ASD symptoms, such as overly repetitive behavior and lack of social skills. Using three-chambered tests to explore the mice's mobility and socialization tests by using other mice they discovered that cannabinoids can be used to treat the symptoms of ASD. A clinical study was also done to test cannabinoids on human ASD patients, related results of cannabinoids having positive outcomes for the patients occurred. The studies all found that combinations of CBD and THC with THC being at lower levels works better than the cannabinoids on their own. Lower levels of THC are needed as it can negatively affect memory for younger children later in their lifetime at higher doses. The future of medical cannabinoids being used to regulate the Endocannabinoid system in Autistic Spectrum Disorder Patients is promising and is gaining popularity as all sources of this thesis are from the last four years alone. The data is highly supported through clinical and animal studies, through continued research with more humans and several types of cannabinoids researchers can get a better understanding of the ECS in ASD and help to attack the cause of symptoms in ASD patients rather than the symptoms alone.

INTRODUCTION

Autism Spectrum Disorder (ASD) is a developmental disability caused by numerous mechanisms in the brain, while the specific cause is unknown there is much research being done on mechanisms including dysregulations of the immune system, endocannabinoid system and overall brain (Nez Govorova 2021, Pol 2021). ASD is multifactorial with varied factors combining to generate the range and severity of symptoms. ASD is one of the most difficult disorders to diagnose as it is found to have a wide range of symptoms that make up the disorder and is different for every patient with ASD. The main symptoms include social impairments, communication and language deficits, seizures, hyperactive or impulsive behavior, unusual eating and disrupted sleeping habits, and unstable moods (Agarwal 2019, Araujo 2019, Carbone 2021, Pietropaolo 2022). There is currently no cure for ASD, rather antipsychotic drugs which make patients feel flat and mask their personalities while only minimizing the symptoms rather than eliminating them (Agarwal 2019, Araujo 2019). They target symptoms such as anxiety, irritability, aggression, and epilepsy. Therefore, the current medications improve the quality of life but are not enough to bring the patient into a normal type of behavior pattern. In contrast for young children with ASD the antipsychotic medication can have extreme adverse effects; weight gain, breast swelling, drooling, tremors, drowsiness, increased heart rate, and in long term heart problems (Mostafavi 2020, Nez Govorova 2021). ASD is found in about 1 in every 100 children and about 10-15 out of every 1,000 adults (Pol 2021). It is more common in males than females at a 3:1 ratio (Pol 2021). ASD is difficult to diagnose for the multitude of symptom's range and severity within ASD and the range they vary from person to person. ASD has undergone the highest number of major changes of definition and symptoms that signify an ASD diagnosis in recent decades from different volumes of The Diagnostic and Statistical Manual of Mental

Disorders (DSM-5) (Araujo 2019, Carbone 2021, Pietropaolo 2022). It was once believed to be only found in adults but more recently found in children.

The current need to help find a specific cause of ASD is based on the need for better medication that treats the cause rather than the symptoms themselves. This can be done through animal research that models ASD symptoms to identify molecular intervention targets through the dissections of ASD mechanisms such as the Endocannabinoid System (ECS). Like the main issue with solving ASD, it is difficult for researchers to find an accurate model in animals due to the range of symptoms and their severity leading to difficulty in reproduction in a single animal model system. (Pietropaolo 2022, Pietropaolo 2020). If there is an animal that displays one symptom but not all, it can be seen as not representing the full disorder and results. The most common animal model is found in mice. Researchers focus on the main core symptoms displayed the most in ASD patients; repetitive behavior, social dysfunction, and lack of communication. Mouse models for ASD and the ECS vary depending on the experiment being run. A study done by Pietropaolo and their colleagues determined different strains of cannabinoids and how they affect the ECS. The best mouse model to date for testing strains is the BTBR T+Itpr3tf/J (BTBR) strain of mice (Pietropaolo 2022, Pietropaolo 2020). These mice were discovered by LC Dunn in the 1950s and inbred them at Columbia University. They have smaller hippocampal commissure than connects their hippocampus halves as well as smaller corpus callosum (Pietropaolo 2022). The hippocampus helps memory functions, ASD patients have a memory deficit and could be found to connect with the hippocampus in BTBR mice. These mice display several abnormalities in behaviors and immune system uptake. Due to the alterations, they show deficits in social interaction, vocalization, grooming, social interaction, and repetitive behaviors like ASD symptoms. Another common mouse model used is Shank3

mice. These mice have 22q13.3 deletion syndrome (Phelan-McDavid Syndrome), a mental retardation syndrome with similar autistic traits. Shank3 mice with mutations also follow autistic pathology. SHANK3 refers to the gene and is different from Shank3 referring to the mouse strain with that gene mutation. The gene is a leading autism candidate gene that encodes protein for proper synapse function and is present in Shank3 mice. (Pietropaolo 2022, Pietropaolo 2020). They also have been found to have changes in their S-nitroso-proteome and can cause dysregulation of key proteins that are used in vesicle release and synaptic function (Pietropaolo 2020). These mice are a similar basis as they express the same social deficit and high repetitive self-grooming behaviors as the BTBR mice. Mice studies are used to find similar symptoms compared to ASD and test different methods of altering the behaviors. Most common for ASD models, scientists will use time spent grooming for mice in hopes that the dependent variable will cause grooming time to decrease. Similarly, they will test sociability by introducing mice to other mice in a cage and reassociating with either a new mouse or the previously introduced mouse; their intended outcome is for the treated mice to be more sociable with the new mouse. The other main experiment is placing the mice in a three chambered field in hopes for the treated mouse to travel more than the placebo mouse. The three chambered test is used to assess cognition by general sociability in rodent models. There is an empty chamber and a chamber with another mouse and a middle chamber, all three are connected by tunnels and the mice explore or there is a new mouse in each of the two chambers. Usually, mice are likely to spend a lot of time with the stranger mouse but with ASD they tend to avoid the new mouse and stay in the chamber with either no mouse or a familiar mouse. Overall, mouse models are responding positively to medical cannabinoid treatments resulting in decreased grooming time and more sociable mice.

The Endocannabinoid system is composed of endocannabinoids; lipid-based neurotransmitters that bind to cannabinoid receptors (CBRs) and cannabinoid receptor proteins that are expressed throughout the central nervous system. It regulates homeostasis of the central and peripheral mechanisms of food intake, lipids synthesis and metabolism in muscle cells (Alexandre 2019, Dasran 2022, Su 2021, Finn 2102). Cannabinoids are chemical compounds that act on cannabinoid receptors and alter neurotransmitter release and/or activity in the endocannabinoid system in the brain. They target cannabinoid receptors and create biological effects. Endogenous cannabinoids are found in the body including Anandamide (AEA) or 2-Arachidonoylglycerol (2-Ag). Exogenous cannabinoids are extracted from the cannabis plant and Hemp. CBD is from Hemp and THC is from the cannabis plant, they elicit euphoric effects through the body including the immune and nervous system. Cannabinoids include Cannabidiol (CBD), Tetrahydrocannabinol (THC), Cannabigerol (CBG), Cannabichromene (CBC), and Cannabidvarian (CBDV) (Burggren 2019, Finn 2021). Cannabinoids have widespread actions in the brain; THC can impair learning and memory through alterations in the hippocampus and CBD can modulate locomotor activity and reward pathways in the basal ganglia which is responsible for motor function. CBD is an antipsychotic that is used for anti-anxiety, neuroprotective, pain relief and anti-inflammation (Agarwal 2019, Alexandre 2019, Aran 2021, Burggren 2019, Finn 2021, Nez Govorova 2021). Antipsychotics reduce delusions, paranoia, anxiety, and agitation. CBD can alleviate pain by reducing inflammation, endocannabinoid receptor activity and interacting with neurotransmitters. THC is a psycho-active and is used for euphoria, anxiety, pain relief, relaxation, and sleep (Agarwal 2019, Alexandre 2019, Aran 2021, Burggren 2019, Finn 2021, Nez Govorova 2021). Cannabinoids can have possible adverse effects; vomiting, agitation, rapid heart rate, hallucinations, and confusion. In serious cases,

synthetic cannabinoid toxicity can be possible which includes acute psychosis, agitation and seizures and sedation. Although rare, Cannabinoid Hyperemesis syndrome is a possible adverse effect due to long term use with side effects including vomiting or nausea and abdominal pain following the use of cannabis (Alexandre 2019, Burggren 2019). Overall, cannabinoids have numerous therapeutic effects including euphoria, enhancement of sensory and perception, as well as can lead to adverse effects including cannabinoid Hyperemesis syndrome and synthetic cannabinoid toxicity (Alexandre 2019, Burggren 2019).

The ECS is regulated by two different receptors; CB1 and CB2. These receptors are embedded in the membrane of cells. The CB1 receptor is found in the brain and central nervous system primarily the hippocampus, basal ganglia, and neocortex. It targets appetite, immune cells, motor activity, motor coordination, pain reception, short-term memory and thinking to overall maintain homeostasis within the body (Su 2021, Behl 2022, Haspula 2020). CB1 is the primary receptor for Anandamide (AEA) signaling; AEA is an endocannabinoid or neurotransmitter that stimulates happiness and euphoria. The CB2 receptor is found mostly in the peripheral organs, especially in immune system cells. It is expressed in adipose tissue, bone, cardiovascular system, central nervous system, eyes, gut, immune system, kidneys, liver, pancreas, reproductive system, respiratory tract, skeletal muscle, skin, and tumors, creating homeostasis (Su 2021, Behl 2022, Haspula 2020). The ECS is made up of endocannabinoid ligands, enzymes that degrade and synthesize endocannabinoid ligands and receptors. The cannabinoids can be either found in the body including Anandamide (AEA) or 2-Arachidonoylglycerol (2-Ag) or cannabinoids from outside of the body including Cannabidiol (CBD) and Tetrahydrocannabinol (THC). The enzymes involved are Fatty acid amide hydrolase (FAAH) which hydrolyzes AEA and related amidated signaling lipids and Monoacylglycerol

lipase (MAGL); hydrolyzes 2-AG and regulates arachidonic acid and prostaglandin production. FAAH and MAGL help break down endocannabinoids like the enzymatic breakdown of neurotransmitters at other synapses (Agarwal 2019, Alexandre 2019, Behl 2022, Pol 2021, Su 2021). Finally, the receptors are CB1 and CB2 as explained earlier. The ECS regulates homeostasis by using a lock and key model, the cannabinoids will lock into the specific receptor on the presynaptic side of the neuron and will signal for a decrease or increase of the neurotransmitter that binds to the postsynaptic cell and triggers a new signal that moves down the neuron processes to the rest of the body (Behl 2022, Dasram 2022, Pol 2021).

The endocannabinoid system in autism is dysregulated. It has been found that patients with Autism have a lower CB1 receptor expression and results in a lack of homeostasis of appetite, memory, seizures, and social mobility (Argarwal 2019). Postsynaptic neurons release endocannabinoids to regulate signaling from the presynaptic cell and are signaling messengers, by activation of the CB1 receptor in the presynaptic neuron there is a decreased release of neurotransmitters to the synaptic cleft and attenuate brain circuits. In ASD it has been found that reduced endocannabinoids are associated in several animal models such as VPA mice and that the activation of the ECS can reverse ASD symptoms (Aran 2021). Similarly, the dysregulation of the Endocannabinoid system can lead to the pathogenesis of epilepsy by causing an imbalance of neurotransmitters; there is now FDA approved for a cannabinoid-based treatment for epilepsy.

Isolated Medical cannabinoids can be used to help regulate the endocannabinoid system in autistic patients. This is due to their ability to mimic the endogenous cannabinoids; AEA and 2-AG and taking their place in the neuron synaptic gap; THC and CBD are released and bind to the CB1 and CB2 receptors (Alexandre 2019). The main difference is that the AEA and 2-AG

are produced by the body whereas THC and CBD are introduced to the brain through orally eating or inhaling.

While the endocannabinoid system is a newly identified system under review with use of ASD, there is research for the therapeutic use of medical cannabinoids to aid ASD patients in finding a medicine that targets the cause rather than the symptoms. This is done by medical cannabinoids to elicit the same anti-inflammatory and anti-epileptic effects as AEA, and 2-AG does. Therefore, due to the dysregulation of the natural endocannabinoids in the ECS in ASD patients, the use of medical cannabinoids may help to regulate the effects that are sent through the neurotransmitters into the body to regulate homeostasis or in patients with autism, help to relieve some of the major symptoms.

Autistic Spectrum Disorder (ASD) is one of the most common behavioral disorders. It causes the most years lost in life span in relation to disorders. New research has found that the main cause of behaviors in ASD patients is the dysregulation of CB1 and CB2 receptor expression in the Endocannabinoid system (Araujo 2019, Colizzi 2022, Haspula 2020, Zamberletti, 2019). The endocannabinoid system is made up of microglia, neurons, receptors, and different hormones. There is an underexpression of cannabinoids to the CB1 receptor that causes inflammatory responses and causes the seizures and behavioral issues found in patients. Cannabinoids with mixtures of CBD and THC ratios have been found to regulate the receptor to normal speed and reduce the inflammation and overall relieving the symptoms. This is done by replacing the lack of endogenous cannabinoids with exogenous cannabinoids to create normal expression of the CB1 receptor. There are experiments being performed on mouse models supporting this idea. This thesis will explore the Endocannabinoid system and target the

receptors using mixtures of cannabinoids to alleviate symptoms and improve the overall life for Autistic Spectrum Disorder Patients.

CURRENT INVESTIGATIONS

There is no cure for Autism Spectrum Disorder (ASD). ASD is a neurodevelopmental disease with core symptoms being deficits in social communication, repetitive behavior patterns, obsessive interests or activities, and intellectual disabilities. Recent studies have investigated the possibility of the Endocannabinoid system playing a role due to a dysregulation of the CB1 receptors in ASD patients, which may contribute to the behavioral symptoms. These receptors can be responsible for many of the symptoms relating to the central nervous system. Medical exogenous cannabinoids such as Avidekel oil are being used to regulate the cannabinoids in the brain and regulate the expression of the CB1 receptor.

Recent Studies are looking into the mixtures of Cannabinoids (CBD) and Tetrahydrocannabinol (THC) within mouse and human studies to alleviate some of the common ASD symptoms; repetitive behavior, seizures, social deficits, and motor impairments.

This study done by Poleg and their colleagues; Kourieh, Ruban, Shapira, Barak and Offen (Poleg, 2021), analyze the use of the Endocannabinoid System (ECS) in Shank3 mice by using Phyto cannabinoids to alter the Na+ concentrations that drive expression of AEA and 2-AG in combination with the CB1 Receptor to overall to alleviate multiple ASD core symptoms. Current research is being done by these researchers and numerous others on the effects of Medical Cannabis with different concentration levels of THC and CBD on patients with ASD. There is evidence for a therapeutic effect and decrease in symptoms for ASD patients with the use of Medical Cannabis, specifically in this article focusing on Avidekel oil which is a concentration of CBD and THC at a 20:1 ratio resuspended in olive oil. Their study found that this Avidekel relieved anxiety and reduced the repetitive grooming behavior in Shank2 mice and could be researched further to aid in ASD studies.

The study uses human mutation based Shank3 mice modeled on ASD to find the connection between interactions of THC and CBD on the Endocannabinoid system contributing to ASD symptoms. Shank3 mice have a mental retardation syndrome that mimics ASD traits such as repetitive behavior and lack of social skills, which make them good test subjects. The researchers are looking into the effects of long-term oral treatment with CBD-enriched medical cannabis oil; in this study the Avidekel oil, both behavioral and biochemical. They hypothesized that CBD and THC enrichment of medical cannabis could help treat core symptoms of ASD by alleviating the deficits of social and repetitive behavior found in SHANK3 mice. The researchers used Shank3 mutant mice which as described earlier and have a deficit in glutamate (a neurotransmitter) production that is used to start synaptic processes that the ECS requires. This deficit results in similar ASD behaviors. The researchers had two cohorts, in the first cohort the experimental group received 5 ml/kg Avidekel oil with a 20:1 CBD:THC ratio, 20 mg/kg CBD, and 1 mg/kg THC. The control group received 5 ml/kg of olive oil. The Avidekel oil was suspended in olive oil to be a proper vehicle in this experiment. In the second experiment naive Shank3 mutant mice were treated with one of four doses; 5 ml/kg CBD oil (25 mg/kg CBD), Erez oil (1 mg/kg THC), THC oil (1 mg/kg THC), or olive oil at 5 ml/kg. The different oils were used to compare the effects of CBD alone, THC alone, olive oil as a vehicle, and CBD/THC together. The Avidekel Oil was a mixture, the Erez oil was a pure THC mixture, the THC oil was a pure THC mixture and finally the olive oil was a pure olive oil mixture used as the placebo. The mice were treated orally twice a week for three weeks an hour before the behavioral tests. Specifically for the grooming test, 30 minutes prior the mice were given an injection of 3 mg/kg of the CB1R antagonist (AM-251) or 0.5 mg/kg of the CB1R agonist (WIN55,212-2). Numerous tests were performed; behavioral tests at 9 weeks old an hour after oral treatment then

biochemical analysis of brain samples at 12 weeks old after euthanasia. HPLC-FD analysis of glutamate and GABA in CSF was done using high performance liquid chromatography with fluorescence detection, behavioral, and evaluation of cannabinoids in serum. Blood samples were collected after terminal bleeding euthanasia of the mice and then incubated at room temperature for 30 minutes after centrifugation at 1000 rcf for 10 minutes at four degrees Celsius. The serum was collected using pipettes and transferred to a 1.5 ml tube for storage at -80 degrees Celsius. Using RNeasy mini kits, researchers extracted RNA from the right cerebellum of the Shank3 mice. The researchers discovered that in compared to the placebo the mice that took Avidekel oil spent half the amount of grooming time, double the time in open arms and a little less distance traveled in the open field during the first cohort whereas in ASD, symptoms included increased grooming time and less time being sociable. The second cohort resulted in significant decreases of grooming time for mice that took Erez and THC oil and an increase of time in social zones for the same mice. CBD showed little to no change from placebo mice on all tests. The results of this experiment were that CBD enriched medical cannabis treatment in InsG3680 Shank3 mouse models of ASD improves repetitive and anxiety behaviors. The ECS process was also expressed using THC-based medical cannabis which alleviates autistic-like phenotypes in mouse models which are mediated by CB1R. The results from the second cohort support this as mice given pure THC oil doubled the time spent in a social zone and decreased the time spent grooming by about 25%. Refer to Appendix, Figure 1. This also explains that CB1 receptors are involved in repetitive behavior alleviation from Avidekel oil treatment. Refer to Appendix, Figure 2.

Overall, this study is looking to help improve the well-being of patients with ASD using a mouse model, the researchers acknowledge the risks and stigma around Cannabis relaying that research efforts are being explored to find a treatment using medical cannabis for individuals

with ASD to alleviate their symptoms and not cause neurological harm at the same time to protect adolescent ASD patients. This study provides an intensive background in the systems of ASD and how cannabis affects these systems to alleviate core symptoms.

Another study by Zamberletti and their associates (Gabaglio, Wooley-Roberts, Bingham, Rubino, Parolaro) have researched the Phyto cannabinoid cannabidivarin (CBDV) treatment in response to Autistic like behaviors in male rats prenatally exposed to valproic acid, or 2-propylpentanoic which is used as an antiepileptic drug and mood stabilizer. VPA consumption during pregnancy can increase chances of ASD in offspring. (Zamberletti, 2019). Valproic Acid (VPA) models displayed common ASD Phenotypes; the researchers believe this to be due to the dysregulations of CB1 receptors in the endocannabinoid system. CBDV is the same as CBD but is an isomer and is only found in extremely small doses. Although CBDV does have similar actions in the body as CBD including pain relief, anti-inflammation, neuroprotective, and anti-anxiety. Valproic acid during pregnancy has had a positive correlation with elevated risk for children to develop features of ASD. Rodents respond similarly to humans when prenatally exposed to VPA, showing increased repetitive behaviors, learning and memory deficit, impaired social interactions and preference for social novelty, and hypersensitivity. Therefore, the study uses rats prenatally exposed to VPA to test the effects of Phyto cannabinoids as a strong model to humans with ASD. Phyto cannabinoids have large pharmacological potential due to their indirect actions on components of the ECS and a range of anti-inflammatory properties. CDBV itself has been found beneficial in Rett Syndrome; a condition that shows overlapping of symptoms with ASD in other studies, to improve motor impairments and cognitive deficits.

The study compares two protocols; a symptomatic treatment in rats compared to a preventative treatment in rats, divided randomly. The goal of the symptomatic treatment is to

assess the ability to counteract autism-like behaviors using CBDV and the preventative treatment was to use CBDV to prevent the appearance of autism-like traits. Their study is on the effects of cannabinoids on the symptoms of ASD and therefore we will not look at the results of the preventive study. The study was done using the male fetus that was treated in the mother's uterus while she was pregnant with 500 mg/kg of valproic acid on gestation day (12.5). The symptomatic group was treated postnatally between days 35-58 with different doses of CBDV (0.2, 2, 20, 100 mg/kg/day). The researchers conducted numerous tests during the study period including behavioral analysis and neurochemical studies. Researchers used 54 control rats from 9 vehicle- treated dams and 75 ASD rats from 15 VPA-treated dams; they used 5-6 rats per liter during four separate experiments.

The behavioral test included a three-chamber test, a NOR test and a repetitive behavior and locomotion test on three consecutive days following PND 30. The three-chamber test was to measure social preference and social approach. Rats were placed in an 80 cm X 31.5 cm x 40 cm arena that had three communicating chambers with central openings that were opened for 5 minutes at a time. The second behavior test to examine social novelty was placing an unfamiliar rat into one of the champers in a wire cage and they could explore for another 5 minutes. The NOR behavioral test used an open-field box with two objects placed in and allowed to be explored for 5 minutes by the rats. After a 3-minute interval one of the familiar objects was replaced with an unfamiliar one, two researchers blind to the experiment collected data on the time spent exploring the familiar object and the new object. The last behavioral test examined the locomotor activity in an activity cage where repetitive behavior was measured by blind observers. The cage was cleaned between each animal and tested using 0.1% acetic acid.

The biochemical studies the researchers conducted included Western blotting and Immunohistochemistry. All animals were euthanized 24 hours after their last CBDV injection, and their brain tissue was randomly assigned to different biochemical analysis procedures. For Western blot analysis the prefrontal cortex and hippocampus were dissected, frozen in liquid nitrogen at -80 degrees Celsius. The immunohistochemistry brain samples were post-fixed in 4% paraformaldehyde in 100mM phosphate buffer pH7.4 and stored in fixative for 48 hours and then kept in 30% sucrose for 24 hours. The rats in the symptomatic CBDV treatment were found to have no side preference within the area for the three-chamber test. Autistic traits would have a specific preferred side due to need for repetition and similar environment found in ASD traits. Although the VPA rats showed a decreased percentage of time spent compared to control rats when exploring a random new rat in a cage into one of the chambers. The researchers found CBDV doses of 20 and 100 mg/kg significantly restored the impairment in sociability in VPA rats. In the social novelty test with comparison of a new rat and rat previously exposed, the study found that VPA rats treated with CBDV 20 mg/kg have reversed the deficit in social preference, where VPA rats avoided the new rat and CBDV rats had interacted with the new rat. The NOR test displayed the reversed effects of short-term memory deficit in male VPA rats at doses of CBDV 2, 20, and 100 mg/kg. The memory deficit was at 81% reduction in VPA male rats originally. The self-grooming and locomotion test discovered a 121% increase in compulsive self-grooming for VPA male rats, CBDV doses of 20 mg/kg significantly normalized the repetitive behavior times. Similarly, the 2 and 100 mg/kg also showed a trend to improve grooming behavior. The CBDV doses of 2, 20 and 100 mg/kg normalized the locomotion by 69% compared to the control group. Refer to Appendix, Figure 3. The biochemistry test done for the symptomatic CBDV rats found that CB1 receptor levels were significantly increased in

prenatal VPA exposure and CBDV rats given the 20 mg/kg dose significantly normalized the receptor expression. This study displays a direct correlation of ASD-like symptoms being normalized with the use of cannabinoids and links the ECS to ASD using VPA rats that display similar behaviors.

The overall goal of mouse trials is in hopes to mimic human models without testing humans first. A large advancement was done by Fleury-Teixeira and their colleagues (Caixeta F.V., Ramires da Silva L.C., Brasil-Neto J.P., and Malcher-Lopes R.) as of 2019 had human trials for effectiveness of CBD-enriched cannabis sativa on autism spectrum disorder (Fleury-Teixeira, 2019). This study had a cohort of 18 ASD patients between ages 6-17 years, 5 females and 13 males. 3 of the 18 patients had left the study after 1 month due to adverse effects, such as nausea or increased appetite. The researchers believe these effects were due to a combination of multiple antipsychotic medications interacting with the CBD. The treatment was based on the severity of symptoms for each patient and given as an oral capsule containing 25 or 50 mg of CBD and 0.34 or 0.68 mg of THC. The initial dose of CBD was 2.90 mg/kg/day and varied on a scale of 2.30-3.60 mg/kg/day depending on severity. The average dose of CBD given from then until the study's completion was 4.55 mg/kg/day with a range of 3.75-6.45 mg/kg/day. The average dose of THC was 0.06 mg/kg/day with a range of 0.05- 0.09 mg/kg/day.

The study results were collected from patients' parents or caregivers using a survey with a score from 0 (lowest level of performance/ maximum level of deficit in symptom) to 100 (highest level of performance/ lowest level of deficit in symptom). A secondary questionnaire was given to determine the degree of change of symptoms in relation to the previous month. A 5-level Likert-like scale was used. A Likert-like scale uses a question or statement followed by a

series of answer statements that respondents choose from to best describe the way they feel, or in this study their child feels to measure attitudes, behaviors, or opinions.

The study does not account for the 3 patients which withdrew but explains reasons for adverse effects for each specific patient. In two of the three the adverse effects could have been due to the unsupervised attempt to reduce or remove the dosages of antipsychotic medication, the third may have suffered adverse effects to the CBD due to two other medications they were taking simultaneously. No differences were found between genders. Of the remaining patients (15), 14 of them (93%) showed improvements in at least one symptom category by 30% or more. The symptoms overall included Attention Deficit/ hyperactivity Disorder (ADHD), Behavioral Disorders (BD), Motor Deficits (MD), Autonomy Deficits (AD), Communication and Social Interaction Deficits (CSID), Cognitive Deficits (CD), sleep disorders (SD), and Seizures (SZ). Seven patients (46%) had 30% or more improvement in four or more symptom categories, two patients (13%) in two symptom categories and five patients (33%) in one symptom category. Only one patient (6%) presented worsening symptoms who was receiving multiple neuropsychiatric medications throughout the study which could have contributed to the adverse effects. The most positive effects were found for Attention deficit/hyperactivity disorder, sleep disorders and communication/ social interaction deficits. The effectiveness for seizures was most impressive, the 5 epileptic patients had a reduction of 50% in three cases and 100% in the other two. Refer to Appendix, Figure 4. Finally, it is important to recognize that most patients; eight out of 10; were able to reduce or discontinue the use of neuropsychiatric medications. This study displays the main purpose of this paper and the purpose of medicine in general. The ability to improve quality of life for others, specifically Autistic Spectrum Disorder patients. This study

was not a discussion of the process of cannabinoids in the brain and the effects of cannabis on Autistic Patients.

DISCUSSION

Interest has grown in the usage of Medical Cannabis for therapeutic use in numerous disorders and diseases. The increasing interest has intersected with autism spectrum disorder and the possible therapeutic effects Cannabinoids can have on the symptoms. Clinical and experimental studies have strongly displayed the positive effects of medical cannabinoids, specifically CBD and THC combinations, on the endocannabinoid system by upregulating CB1 receptor binding in Autistic Spectrum Disorder patients and alleviating symptoms related. This research is focusing on targeting the cause of the symptoms rather than the symptoms themselves.

ASD symptoms that are tested for are most commonly in rodent studies involving time spent grooming, social interactions, and repetitive behavior (Poleg 2021). ASD mice have been found to have increased time devoted to repetitive behavior, lack of social interactions, and decreased increased grooming time, like humans diagnosed with ASD who have multiple repetitive behavior patterns, lack social skills to communicate with others, and the decreased grooming time correlates with their instincts of self-care. Mouse studies have shown that the benefits of a tincture of CBD and THC with a low ratio of THC are significant, including regulating CB1 receptors, eliminating symptoms of ASD and helping increase quality of life for ASD patients (Zamberletti 2019). THC has been shown to have negative effects such as memory deficits later in life on younger patients and therefore found to be at lower concentrations in studies for ASD, especially in children. Higher ratios of CBD can attenuate the THC psychotropic effects (Zamberletti 2019).

The use of different mixtures of CBD and THC were found to have important effects on ASD symptoms in Shank3 mice with Poleg and their colleagues. With the most promising results

in grooming time and social ability which are both at a deficit in ASD patients. While the placebo mice that received only olive oil and no cannabinoids had no effect on both mixtures; Avidekel (20:1 CBD: THC) and Erez (25:1 CBD: THC), had significant results by decreasing grooming time and increasing time spent in the social zone in Shank3 mice. These results can lead to a discussion of different concentrations of doses for CBD and THC and proof for a positive change in ASD patients. The mixtures of CBD and THC can be varied to find the best tincture for each patient specifically (Poleg 2020).

Using the Valproic Acid (VPA) rat model which shows common ASD symptoms, researchers can determine if counteraction for autistic behaviors is possible using CBDV. The rats in the symptomatic cannabinoid; Cannabidvian (CBDV) treatment were found to have no side preference within the area for the three-chamber test. The symptomatic treatment was to use cannabinoids to help reverse ASD symptoms rather than prevent them. Autistic traits would have a specific preferred side due to need for repetition and similar environment found in ASD traits, this result leads to the idea that CBDV can help reduce the autistic symptoms and return the VPA mice to a more normal behavior. The researchers finding the highest CBDV doses of 20 and 100 mg/kg significantly restored the impairment in sociability and in the social novelty test with comparison of a new rat and rat previously exposed, the study found that VPA rats treated with CBDV 20 mg/kg have reversed the deficit in social preference (Zamberletti 2019). These results can lead to a discussion on the diverse levels and combinations needed to reverse Autistic Symptoms as in this case only two of the five doses made significant changes. The VPA mouse model shows significant possibility of being a model for ASD as it was found to have a 121% increase in compulsive self-grooming, a trait of ASD patients. The NOR test and biochemistry results of the VPA rats show a return of CB1 receptor expression levels from overactive in VPA

rats to normal with CBDV treatment and shows a significant return of short-term memory from 81% reduction in VPA rats to normal after treatment. Returning to normal receptor expression levels gives significant data on the use of cannabinoids to return the ECS back to normal functioning which in turn will help return ASD symptoms back to regular behaviors (Zamberletti 2019).

Clinical studies can lead to a more comprehensive understanding of the effects of medical cannabinoids on ASD symptoms. Fleury-Teixeria and their colleagues created a study with data supporting the theory of cannabinoids being used to treat ASD. With all epileptic patients having 50-100% reduction of seizures, due to the concentration of 20:1 (CBD: THC), there is support of effectiveness for cannabinoids to reduce epileptic symptoms in ASD patients (Fleury-Teixeira 2019). Similarly, most of the main symptoms of ASD including deficits in communication, social interactions, sleep, and ADHD improved behaviors for ASD patients after use of cannabinoids. This is alleviating their symptoms and bettering the overall life experience for patients. This study also explores the idea of mixed concentrations of CBD and THC for the best results which can contribute to the future research of different dosage levels (Fleury-Teixeira 2019).

In the coming future, researchers should be looking into experiments testing different strains with different concentrations of THC and CBD with specifically lower THC concentrations for children patients to avoid adverse effects in later life. Rather than tests on CBD and THC separately, studies should focus solely on the combinations at different tinctures to determine what mixtures work best for the different systems. This could help create specified medical treatment plans personalized for each patient as not all patients have the same symptoms with similar severity. The treatment of ASD could be found to be on a patient-to-patient level rather than overall. Similarly, different mouse strains can be experimented with as there are

numerous that loosely fit into an ASD model, this could determine the strength of the claim that dysregulation of the Endocannabinoid system may be a cause for the symptoms of ASD. Clinical human studies could also lead to a better understanding of the long-term effects of medical cannabinoids on ASD patients, especially children. Clinical studies also need to move forward as most work is currently on mice models that can mirror the ASD symptoms in ASD human patients, but human studies can provide more accurate results on the possibility of cannabinoids aiding in the regulation of the ECS.

The future of medical cannabinoids being used to regulate the Endocannabinoid system in Autistic Spectrum Disorder Patients is promising and is gaining popularity as all sources of this thesis are from the last four years alone. In hopes to increase the overall life experience for ASD patients, studies need to look at the potential causes rather than the symptoms alone. Exogenous cannabinoids such as CDB and THC, specifically mixtures of CBD and THC at low THC concentrations have significant possibilities to replace the lack of endocannabinoids in the Endocannabinoid system and regulate CB1 receptor expression to regulate ASD symptoms.

APPENDIX

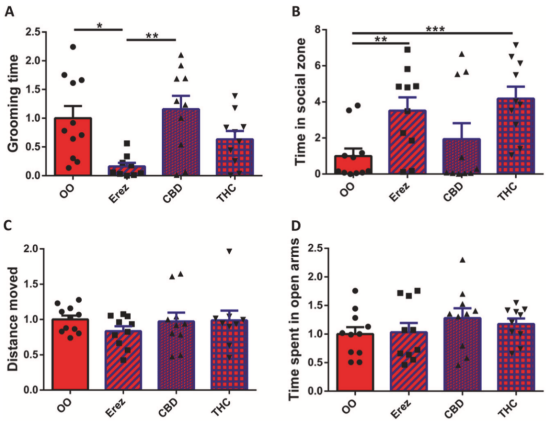

Figure 1: Shows the mechanism for Avidekel oil in the INsG3680 Shank3 mouse model of ASD. Comparison of olive oil and ADKL on grooming behavior that displays a significant decrease in grooming time, an increase of time spent in open arms, and time spent in social zone for ADKL oil mice. Figure 1 is reproduced from Poleg 2021, Figure 3.

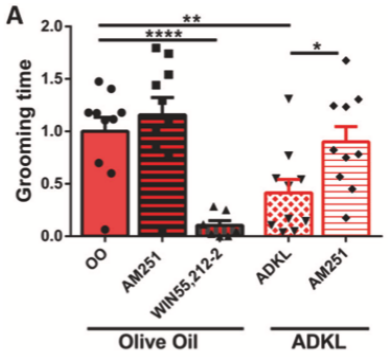

Figure 2: Displays that CBD is not the sole factor for improvement of ASD symptoms. 2A. Significance of Erez and THC to decrease grooming time. 2B. Significance of Erez and THC on social improvements. 2C,2D. No difference between oral treatments for distance moved and time in open areas. Figure 2 is reproduced from Poleg 2021, Figure 5.

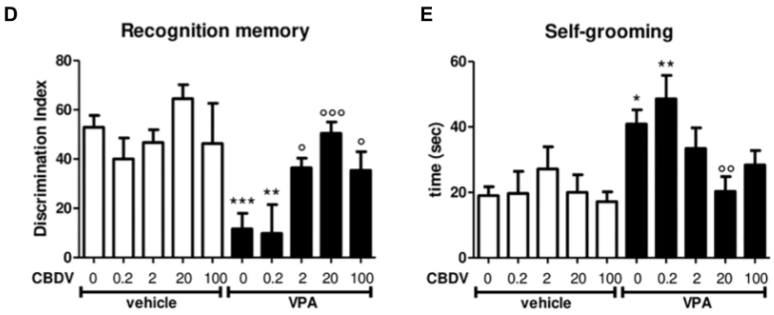

Figure 3: 3D displays memory deficits being returned due to CBDV treatment. 3E shows decline in grooming time for CBDV treated rats. Figure 3 is reproduced from Zamberletti, 2019 Figure 1.

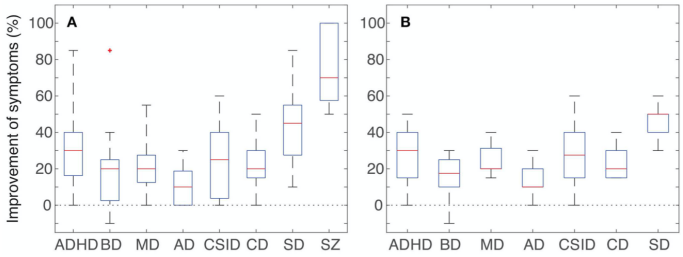

Figure 4: Improvement of symptoms in percentages A) In epileptic patients B) Non-epileptic patients. With improvements in all but Autonomy Defects (AD) in epileptic patients.

Figure 4 is reproduced from Figure 1 from Fleury-Teixeira, 2019.

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