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Review

Autism and associated disorders: cannabis as a potential therapy

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Abstract

Autism spectrum disorder (ASD) is a group of disabilities with impairments in physical, verbal, and behavior areas. Regardless the growing frequency of autism, no medicine has been formed for the management of the ASD primary symptoms. The most frequently prescribed drugs are off-label. Therefore, there is necessity for an advance tactic for the treatment of autism. The endocannabinoid system has a central role in ruling emotion and social behaviors. Dysfunctions of the system donate to the behavioral deficits in autism. Therefore, the endocannabinoid system represents a potential target for the development of a novel autism therapy. Cannabis and associated compounds have produced substantial research attention as a capable therapy in neurobehavioral and neurological syndromes. In this review we examine the potential benefits of medical cannabis and related compounds in the treatment of ASD and concurrent disorders.

Keywords: Autism; Endocannabinoid system; Medical cannabis; CBD; THC; CBDV

1. Introduction

Autism spectrum disorders is neurodevelopment disorders with wide range of impairments in social communication and restricted and repetitive behaviors [1]. The intellectual capability of individuals with ASDs is highly variable and ranges from severe impairment to superior performance. Both, Autism and ASD are used interchangeably. In the USA, the prevalence of ASD is approximately 4.5 times greater in boys than in girls [2]. ASD occurs in all racial, ethnic, and socioeconomic groups, although white children are more likely to be diagnosed with ASD than black or Hispanic [1]. According to the WHO, on a worldwide basis, it is estimated that 1 in 160 children exhibit ASD [3].

Autism is a behavioral diagnosis, the exact cause of which is unknown and currently biomarkers have not been identified. Several factors including environmental, biological and genetics play a role in the pathogenesis of ASD. Approximately 15–20% of ASD cases were found to be associated with genetic mutations [4]. Fragile X mental retardation 1 (FMR1) is the most common single gene mutation identified in autistic individuals [5–9]. Other single gene mutations that have been shown to be associated with ASD include tuberous sclerosis, neurofibromatosis, Angelman syndrome, and Rett syndrome [5,10]. Immune dysfunction and inflammation as well as fetal exposure to antiepileptic drugs contribute to the pathogenesis of autism [11–17].

The endocannabinoid system (ECS) has been investigated for its association with ASD because of its role in regulating emotion and social behaviors. The endocannabinoid system involves the cannabinoid receptors (CB1 and CB2) and their endogenous ligands (the endocannabinoids) as well as the enzymes involved in the biosynthesis and inactivation of the endocannabinoids [18]. The primary endocannabinoids are N-arachidonoyl-ethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG). Enzymes involved in the synthesis of AEA and 2-AG are N-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD) and diacylglycerol lipase (DAGL), respectively. Endocannabinoids are inactivated by hydrolytic enzymes, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Inhibitors of FAAH and MAGL may influence signaling of ECS. The endocannabinoids act as retrograde messengers on presynaptic cannabinoid receptors to lower the release of neurotransmitters (e.g., monoamine, opioids, GABA, glutamate, acetylcholine) and to impact a wide range of biological processes [18].

Several studies have suggested that dysfunctions in the components of the endocannabinoid system may contribute to the behavioral deficits and neuroinflammation observed in autism [19–24]. In animal models of ASD, modulation of the endocannabinoid system has been shown to improve certain ASD-associated social and cognitive impairments [25–28].

Although the prevalence of autism is increasing [29], a pharmaceutical has not been developed for the treatment of the core symptoms of ASD. Management of ASD calls for a multidisciplinary approach and mainly involves behavioral and educational interventions [1,30,31]. Pharmacological therapy attempts to address ASD-associated comorbidities including seizure, violent behavior, psychosis,

anxiety, depression, bipolar disorder, and attention-deficit hyperactivity disorder [30–32]. The most frequently prescribed classes of drugs for ASD patients include antidepressants, stimulants, antipsychotics, anticonvulsants, hypotensive agents, anxiolytics/sedatives/hypnotics, and melatonin [33–35].

Marijuana is regulated as a schedule 1 substance by the US federal government. However, 36 states, the District of Columbia, Guam, Puerto Rico, and the U.S. Virgin Islands have comprehensive medical cannabis programs. In addition, the remaining 14 states allow use of cannabidiol (CBD) with minimal or no delta-9-tetrahydrocannabinol (THC) for medical reasons in limited situations [36]. Among these, 12 states approved cannabis for the treatment of ASD and self-injurious or aggressive autistic behavior [37]. Most of European countries have legalized medical cannabis in recent years. The European Union recently legalized CBD and hemp products. However, some countries legalized only derivatives of the cannabis plant and not flowers or other natural forms of the plant [38].

The medicinal use of cannabis in ancient China dates back to about 2700 BC [39]. According to the US drug enforcement administration (DEA), hemp and cannabis comprise separate parts of the cannabis plant [40]. Three species of cannabis including cannabis sativa, cannabis indica, and cannabis ruderalis have been identified [41–43]. Cannabis contains more than 100 cannabinoids; CBD and THC are the focus of most studies [44,45]. THC is the main psychoactive constituent and is a partial agonist of CB1 and CB2 receptors. THC can produce neuroprotective, analgesic, appetite stimulant, antiemetic, and antiglaucoma effects [46,47]. In contrast to THC, CBD has little affinity for CB1 and CB2 receptors but functions as an indirect antagonist of cannabinoid agonists. CBD is also an inverse agonist of CB2 receptors. CBD can counteract some of the functional consequences of CB1 activation in the brain and exhibits anti-inflammatory, antioxidant, anticonvulsant, and neuroprotective effects and reduces THC psychoactivity [45,48,49]. Other cannabinoids including non-psychoactive cannabidivarin (CBDV) also contribute to the cannabis medicinal effects. Studies in animal models and humans advocated for anti-inflammatory, neuroprotective, anxiolytic, and antipsychotic properties of cannabis compounds.

Here we present a comprehensive review on: (i) Changes in the endocannabinoid system in autism (ii) Effect of cannabis on autism (iii) Effect of cannabis on autism-associated disorders.

2. Changes in the endocannabinoid system in autism

Therapy for autism has been difficult to establish because it is a multifactorial disorder. ASD manifests due to a combination of genetic, immunological, and environmental factors that result in communication and behavioral problems [50–52]. Many studies have shown that the ECS plays a crucial role in regulating emotional and social behavior [53–57]. Dysfunction in the ECS was connected with the pathology of neurodevelopmental disorders, most specifically ASD [24,58]. Pathophysiological mechanisms producing the neurobehavioral deficits in ASD include aberrant synaptic plasticity, immune dysfunction, and metabolic disturbances, all of which are regulated by ECS [14,35].

The ECS represents a complex system of lipid signaling pathways [59,60]. The ECS plays an important role in the development of the central nervous system (CNS) [61–63]. CB1 receptors are located in the central nervous system, peripheral nervous system, and peripheral organs. In the CNS, CB1 receptors are concentrated in the cerebellum, hippocampus, and the basal ganglia, which are areas of dysfunction in autism [64–66]. CB1 receptor activation results in glutamate release, inhibition of synaptic transmission and regulation of synaptogenesis, axonal outgrowth, differentiation, migration, and proliferation [61,67–69].

Autism is also associated with dysregulation of the immune system [15,70,71]. CB2 receptors are located in immune cells and may control the movement of inflammatory cells [64,72–76]. CB2 receptors are also expressed in microglia and astrocytes, which may be critical to ASD-related neuroinflammation [77]. Immune dysfunction, increased autoimmune activity and inflammatory responses are associated with microglial activation in patients with ASD [78]. Microglia activation, increased levels of inflammatory cytokines and chemokines, and augmented expression of the microglial activation-related genes are the results of the pro-inflammatory status of the immune system in autism [78–81]. Moreover, the mRNA and protein for CB2 receptor and endocannabinoid enzymes were significantly changed in animal models of autism, demonstrating the involvement of the ECS in ASD-associated immunological disturbances [52,70]. Studies focused on children with autism have shown alterations of the immune system such as variation in monocyte and macrophage reactions, abnormal T helper cytokine levels, reduced numbers of lymphocytes, and abnormal immunoglobulin levels [82–87]. In addition, an increase in pro-inflammatory cytokines was associated with more regressive forms of autism and more pronounced stereotypical behaviors [78].

Several investigations revealed the involvement of the ECS in neuromodulation as well as in the regulation of emotional responses, behavioral reactivity, and social interaction in ASD [19,24,88]. Disruption of this system may impair social communication, social play, and reciprocity [19]. Some studies investigated the association of acetaminophen with social behaviors and ASD confirmed activation and involvement of the ECS in autism [89–96].

Additional studies have established the connection between the endocannabinoid system and autism. Lowered CB1 receptor level was found in postmortem brains of autistic patients [78]. Stimulation of the CB1 receptors

either directly by the synthetic cannabinoid receptor agonist WIN55212-2, or indirectly by a 2-AG hydrolysis inhibitor, increases the spatial memory performance of rats under stress conditions [97]. Polymorphisms in the gene encoding the CB1 receptor, CNR1, were found to modulate striatal responses and gaze duration to social reward cues [98,99], indicating that changes in endocannabinoid affinity to the CB1 receptors may lead to deficits in social rewards observed in autism. Additionally, the CB1 receptor has neuroprotective capacities manifested by decreasing tumor necrosis factor (TNF)-α levels in neurodegenerative conditions [100].

Animal studies implied that social play behavior boosts AEA levels in several brain regions [55,56,101]. High AEA concentrations, following inhibition of FAAH, result in CB1 receptor activation and improved social play behavior [89,102,103]. Plasma concentrations of AEA were found to be lower in children with ASD compared to healthy controls [18,104]. In addition, FAAH inhibition boosts levels of N-acylethanolamines such as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) in hippocampus. High OEA and PEA levels can compete with AEA for FAAH enzyme and lead to reduced AEA metabolism and enhanced activity of the CB1 receptors [101]. Moreover, as neither OEA nor PEA have affinity for CB1 receptors, it is possible that competition for FAAH forces AEA activity back to the CB1 receptors [19]. This suggests that a deficiency in social play behaviors may be caused by low AEA levels in critical brain areas. However, earlier studies have demonstrated that broad excitation of CB1 receptors interferes with the normal excitation of complex social acts, possibly by interfering with cognitive functions required for normal social interactions [101,103]. Additionally, autism mouse models demonstrate a downregulation of G protein-coupled receptor 55, GPR55 and peroxisome proliferator-activated receptor, PPAR, which can be alternative receptors involved in social play behaviors [19]. Some of the behavioral changes may be mediated by AEA activation of other receptor targets or direct activation of PPARγs by OEA or PEA, since data indicate that activation of hippocampal PPARγ enhances cognitive performance [105]. PEA with its intestinal anti-inflammatory characteristics is a point of interest for autism since part of the autistic chronic inflammatory state is mediated via the gastrointestinal associated immune system [83,106,107]. The observed anti-inflammatory effects of PEA are exerted through activation of CB2, GPR55 and PPARγ receptors [108].

The endocannabinoids through CB1 receptors can reduce glutamate release, alter synaptic plasticity and, therefore, modulate neurotransmission in the basal ganglia. Cannabinoid signaling can release dopamine that counteracts the effects of induction of the CB1 receptor via dopamine D1-like receptors [109]. On the other hand, dopamine signaling via dopamine D2-like receptors may lead to upregulation of endocannabinoid signaling [109]. Endocannabinoid signaling also functions as a retrograde signaling system in GABAergic and glutamatergic synapses [110,111].

Different genetic animal models are used to explain mechanisms of action of the endocannabinoids. The BTBR T+tf/J (BTBR) mouse strain have autism-like phenotype. CB1 density in the BTBR hippocampus is 15–20% higher than in other strains. GTPγS- stimulated binding of CB1 agonist cannabinoid CP55940 to Gi/o-coupled receptors in the BTBR animals is also elevated, indicating a potential for increased sensitivity [112,113]. In BTBR model increased AEA action at CB1 receptors improved social impairment and reduced locomotor movement, implying an influence on irritability and repetitive behaviors [114]. Furthermore, the treatment of BTBR mice with the FAAH inhibitor, URB597, and the cannabinoid delta 9-tetrahydrocannabinol (Δ9-THC) ameliorated the social behavior deficits of BTBR mice [25,114,115]. There is also evidence for elevated CB2 receptor expression in the BTBR mouse brain [116]. Genomic studies revealed an upregulation of mRNA levels of the CB2A isoform, in the cerebellum of BTBR mice [115,116]. A clinical study in young children demonstrated upregulation of CB2 gene expression in peripheral blood mononuclear cells of patients with autism [117]. The increase in CB2 expression may serve a compensatory role for the inflammation associated with autism [118–121]. Therefore, keeping in mind that AEA suppresses the release of proinflammatory cytokines through a CB2-mediated mechanism [77], the enhancement of CB2 may be negative feedback to reduce the proinflammatory responses in ASD. Moreover, in the Fragile X syndrome (FXS) model of autism mRNA levels of the enzymes (DAGL and MAGL), were altered in the cerebellum and hippocampus, whereas levels of 2-AG in the same regions were not changed [55,122]. In the FXC model, treatment with URB597 (selective inhibitor of the FAAH) resulted in an increased AEA activity, improved memory and anxiety-like behavior, and reversed the social impairment [81].

Studies on Shank3B$^{-/-}$ mouse with significant social interaction disorder, have shown that pharmacological augmentation of 2-AG levels by ZL184 (monoacylglycerol lipase inhibitor) normalized social interaction deficits [123]. Mutations in neuroligin-3 gene, 4 (NLGN 3, 4), which is involved in the formation and remodeling of CNS synapses, are associated with intellectual disability, seizures, and autism behaviors [124,125]. Studies in mice with a neuroligin-3 amino acid substitution (R451C) and a neuroligin-3 deletion revealed that neuroligin-3 is specifically required for tonic endocannabinoid signaling, further confirming variations in endocannabinoid signaling can donate to the pathophysiologic mechanism of autism [23,126]. Additionally, it was shown that a synthetic cannabinoid WIN55212-2 may reduce aggressive behavior of neuroligin-3 (NL3) R451C mouse model of ASD by modulation of CB1 receptor [127]. Another animal

model with rats prenatally exposed to valproic acid (VPA), exhibits ASD-like abnormalities in sociability and nociception tests, and alterations of distinct elements of endocannabinoid system [19,128]. VPA rats have lessened level of mRNA of PPARα and GPR55 in hippocampus and frontal cortex, decreased level of FAAH and abnormal AEA activity, favoring ASD like behavioral symptoms [19,128]. It was reported that the FAAH inhibitor PF3845 enhanced AEA signaling and weakened the discrepancy in social behavior in VPA rats [19]. Another inhibitor of FAAH enzyme, URB597 improved social deficits, repetitive behaviors, and abnormal emotion-related behaviors in VPA-exposed offspring [78]. The results were produced by the deletion of postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) subunits GluA1 and GluA2 and helped to characterize the mechanism of AEA signaling in the prefrontal cortex [78]. Moreover, increased 2-AG concentrations developed the improvement of behavior defects in VPA rat model after treatment with JZL184 [18].

Recent testimony clearly links alterations in the ECS with autism. The lowered endocannabinoid levels, degradation of enzymes and CB receptors up-regulation indicate reduced endocannabinoid signaling in ASD. Taken together, the findings lead to the hypothesis that influencing the ECS can normalize different behavioral patterns compromised in ASD and recommend the ECS as a novel target option for autism pharmacotherapy.

3. Cannabis in the treatment of autism

3.1 Preclinical studies

Cannabidiol is effective in the treatment of some neurodevelopmental conditions including ASD. CBD has low affinity for the CB1 and CB2 receptors, but m bind to these receptors. CBD has high affinity for the TRPV1 and the TRPV2 receptors involved in stimulation of the CB1 receptors [61,78,129]. In animals cannabidiol increases the serum levels of AEA by inhibiting the FAAH enzyme and normalizes the depletion of AEA tone and, as a result, improves the ASD symptoms [130,131]. In humans CBD's effect on AEA serum levels may be due to inhibition of the metabolism [132]. Since decreased AEA level produces social impairment in genetic models of ASD, intensifying AEA signaling may inverse the behavioral disorders. The administration of CBD to C57BL/6J mice (model with compulsive behavior) alleviated marble-burying behavior, which is analogous to repetitive and compulsive behaviors observed in ASD [78]. There is also preclinical evidence supporting that CBD improved autism-like social behavior in mice with Dravet syndrome and the social and cognitive dysfunctions in rat model of schizophrenia [133,134]. In murine models CBD modulated immune function by reducing activation of microglia and decreasing expression of chemokine ligands and interleukins [129]. In another study, cannabidiol reduced neuronal excitability and transmission through inflammatory pathways by inhibition of adenosine reuptake and modulation of the release of pro-inflammatory cytokine tumor necrosis factor alpha (TNFα) [135]. In addition, chronic CBD administration rescued several autistic-like behaviors (anxiety- and depression-like behavior, poor social interaction, and increased rearing behavior, as well as reference memory and working memory) in Scn1a$^{+/-}$ mice (Dravet syndrome animal model) [133,136]. CBD did not induce any adverse effects on motor function, giving further support for the benefits and safety of using this cannabinoid as ASD therapy.

THC also can produce a positive effect on the neurodevelopmental conditions such as autism. THC treatment increased locomotor behavior and reduced the depressogenic profile in BTBR mice with an autism-like phenotype [115].

Another widely studied nonpsychotropic phytocannabinoid is cannabidivarin (CBDV). Although the mechanism of actions of CBDV is still unclear, it was reported that CBDV might produce its effects through voltage dependent anion selective channel protein 1 (VDAC1), or through the activation and desensitization of TRPV1 channels [135]. CBDV reduces neuronal excitability and neuronal transmission and may also inhibit adenosine reuptake or modulate the release of pro-inflammatory cytokine tumor necrosis factor alpha (TNFα) [137]. CBDV as TRPV1-antagonist with anti-inflammatory activities, decreases inflammation involving cytokine production [138–140]. A therapeutic potential of CBDV was supported by the certain effects of the cannabinoid in Mecp2 seizure animal model (Rett syndrome model) and VPA model [128,141–143]. CBDV increased the AEA and OEA levels, reduced DAGL-a expression and reduced CB1 and CB2 receptor levels in the Mecp2 mice [144,145]. As a result, CBDV restored the compromised general health status, the behavioral deficit, the sociability, and the brain weight and produced a rescue of memory deficits in this animal model [144,145]. Surprisingly, CBDV restored neurotrophic factor levels and ribosomal protein six phosphorylation in the mice, though both were expected to be impaired in ASD [145]. In a study with VPA rats CBDV repaired hippocampal endocannabinoid signaling and neuroinflammation [146]. This cannabinoid produced an increase in CB1 receptor, FAAH and MAGL levels, enhanced GFAP, CD11b, and TNFα levels and caused microglia activation in the hippocampus [146]. As a result, social impairments, short-term memory deficits, repetitive behaviors and hyperlocomotion were restored [146]. On the other hand, chronic administration of CBDV induced an increase in glial fibrillary acidic protein (GFAP, associated with CNS inflammation) in control and VPA animals [146]. Overall, the ASD-like behavioral changes were repaired by CBDV treatment suggesting that the beneficial effects of CBDV could be related to the restoration of the ECS abnormalities in the hippocampus. The correlation between improvement of the behavioral

deficits and modulation of the ECS was consistent with other studies [78,104,127,128].

These current data strongly link alterations of the ECS with ASD and provide evidence supporting the ability of cannabis or/and certain cannabinoids to improve anomalies similar to core and associated symptoms of ASD. However, the exact mechanisms of action of cannabinoids in ASD patients remains unclear [73,76]. The non-endocannabinoid mechanisms may involve the regulation of glutamatergic and GABAergic neurotransmission and receptors including the GPR55, 5-HT1, $\alpha 3$ and $\alpha 1$ glycine receptors, and TPPA1 channel [56,108,110,111,133]. Moreover, CBD may also indirectly act through neuropeptides such as oxytocin and vasopressin that are involved in social bonding, compromised in ASD [54,81].

3.2 Clinical studies

Cannabis has an extensive range of clinical applications including treatment of multiple sclerosis, Tourette syndrome, Parkinson's disease, epilepsy, glaucoma, nausea, depression and pain [37,59,60,147–151]. Epidiolex® (CBD) has been FDA- and EMA-approved for two epilepsy syndromes related to ASD: Dravet and Lennox-Gastaut Syndrome [149,150]. Autism is associated with disruption of the endocannabinoid system [19,88,152]. Cannabis and some cannabinoids have ability to modulate the ECS and, therefore, improve behavioral deficits in autism.

Use of cannabinoids for autism has a growing interest in social media. A number of anecdotal self-reported cases show that ASD children who failed traditional pharmacologic therapy have responded to cannabis treatment. Parents of these children have reported remarkable improvements. A child with autism has spoken first words after receiving cannabis oil and finally, developed significant language skills [153]. In another case, FDA-approved medications produced life-threatening toxicities in 10-year-old boy with autism. He was subsequently started on cannabis; six years after the six-months-terminal diagnosis, the boy was sociable and successful [153]. Similarly, other boys with severe ASD, treated with various therapies did not result in alleviating autistic symptoms. The boys showed dramatic improvement in communication skills and interactions after they were given cannabis [154–156]. Smoking cannabis improved the sociability, vocabulary and reduced anxiety of a 20-year-old ASD man [157]. Another boy with a brain tumor, autism, severe seizures, and self-destructive behavior was treated with cannabis and showed remarkable progress [158].

Despite the growing interest, there are very limited clinical data on the impact of cannabis on autism. In a prospective single-case-study dronabinol (THC) produced improvement in hyperactivity, irritability, stereotyped behaviors, and speech in an ASD boy [159]. In another open label study dronabinol also produced significant improvements in self-injurious behavior of seven out of 10 mentally retarded adolescents [160].

Positive results in autistic patients treated with cannabis as well as increasing anecdotal reports of cannabis beneficial effects on autistic children have led to more scientific testing. In January 2017, Shaare Zedek Medical Center initiated a phase II clinical trial (NCT02956226) to evaluate the safety and efficacy of cannabis (CBD: THC, 20:1 ratio) in children with autism [161]. This three-month study involved 150 patients (aged 5–21 years) with mild to severe autism. The investigators informed cannabinoid therapy was correlated with reduction in disrupting behavior, the CGI-I (Clinical Global Impression-Improvement) scale improved in 49% patients against 21% control patients. Median SRS (Social Responsiveness Scale) score increased 14.9 points versus 3.6 points on placebo. The therapy produced a decrease in body weight in obese patients. This is particularly important as antipsychotics are linked to substantial weight gain. No significant adverse incidents were reported [162]. Common adverse events included somnolence and decreased appetite, reported for 28% and 25% on whole-plant extract, respectively (n = 95); 23% and 21% on pure-cannabinoids (n = 93), and 8% and 15% on placebo (n = 94). Even the study demonstrated that medical cannabis has the ability to improve ASD disrupting behaviors with adequate acceptability, evidence for efficacy of cannabinoids is insufficient. Further testing was recommended.

More clinical trials were conducted recently. A retrospective study assessed effect of cannabis (CBD:THC, 20:1 ratio) in 60 ASD children with severe behavioral problems [104]. This therapy resulted in improved behavioral outbreaks in 61% patients. Anxiety, communications as well as disruptive behaviors were also improved in 39%, 47%, and 29%, respectively. The Autism Parenting Stress Index reported 33% less stress [104]. Adverse events were sleep disturbances, irritability, and loss of appetite. One patient with a higher THC dose had a brief psychotic event [104]. In an open-label prospective study, 188 ASD patients received cannabis oil with 30% CBD and 1.5% THC (20:1). After six months of treatment, 60.0% of the patients were evaluated. Substantial progress was reported in 30.1%, moderate in 53.7%, minor or no improvement in 6.4% and 8.6% of the patients, respectively. The most common side effect was restlessness [163]. The same CBD:THC ratio (20:1) was used in a prospective study with 53 autistic patients [164]. It was reported that self-injury and rage attacks improved in 67.6% and worsened in 8.8%; hyperactivity better in 68.4% and deteriorated in 2.6%; sleep problems improved in 71.4% and aggravated in 4.7% patients. Adverse effects, sleepiness and alteration of appetite were weak [164]. In a double-blind study with 34 men (half with ASD) CBD amplified fractional amplitude of low-frequency fluctuations (fALFF) in the cerebellar vermis and the right fusiform gyrus and modified vermal functional connectivity in ASD group only [165].

Recently an observational trial (CT03699527) with 200 children with ASD was completed. Goal of this investigation was to assess disposition, pharmacokinetics and pharmacodynamics of medical cannabis products including CBD and to provide evidenced based dosing guidance for these products to the children [78]. Another open clinical trial (NCT03900923) for CBD in youth with ASD (n = 30) was recently announced [166]. Aims of the investigation are to detect the optimal CBD doses and to characterize outcomes for upcoming studies. Another placebo-controlled trial (NCT03537950) examines brain reaction to a single dose of CBD and CBDV in men (n = 38) with and with no autism [167]. A research program at Montefiore Medical Center, Albert Einstein College of Medicine is conducting a 12-week Phase 2 double-blind, randomized, placebo-controlled trial (NCT03202303) with CBDV in 100 children and adolescents with autism [168]. An open-label clinical trial (NCT03849456) investigating CBDV effect in ASD patients was terminated due to enrollment challenges during COVID-19 pandemic [81].

In addition, recent case series studied the impact of cannabis on Fragile X patients. It was reported reductions in social evasion and anxiety, improvements in sleep, motor and language skills after the CBD treatment. Two out of three patients exhibited anxiety reappearance following termination of the therapy. CBD was shown to be a medicinal candidate for FXS patients. However, rigorous clinical trials are required to support this finding [169].

Based on available data cannabis was found as safe and effective choice to alleviate ASD signs. But cannabis produces not only therapeutic effects but also creates dangerous health complications. One major concern is the impact of cannabis on brain. Cannabis impairs normal brain development and may result in potentially non-reversible neurocognitive changes [170]. Early cannabis use was correlated with a significant drop in IQ at age 38 years [171]. Cannabis use has been shown to impair cognitive functions from basic, such as motor coordination to more complex executive functions [172]. Chronic cannabis was associated with cognitive problems such as addiction, distorted perceptions, difficulty in thinking and problem solving, working memory deficits, and abnormal social behavior [152,171–175]. These deficits vary in severity and depend on the quantity, recency, age of onset and duration of cannabis use. Moreover, individuals with cannabis-related impairments have been found to have trouble for successful recovery, putting them at increased risk for relapse to cannabis use. Further, termination of cannabis use did not fully restore neuropsychological functioning among adolescent-onset cannabis users [173]. Cannabis use is also specified as a potential source, aggravator, or masker of psychiatric symptoms particularly in young people [176–179]. It is difficult to recognize patients who benefit and who build side effects [180]. For instance, while cannabis might be beneficial in persons with one phenotype, it may have no effect or severe adverse outcomes in persons with other phenotypes [181]. Therefore, all positive and negative effects of medical cannabis have to be carefully assessed in large studies.

4. Cannabis in the treatment of autism-associated disorders

An exciting hypothesis about the medicinal potential of cannabis is that cannabis/cannabinoids may improve disorders co-occurring with ASD, including ADHD, seizures, anxiety, mood disorders, and sleep disturbances.

4.1 Attention deficit hyperactivity disorder

Attention-deficit hyperactivity disorder (ADHD) and ASD are frequently comorbid, have similar genetics and cognitive deficits [182,183]. ADHD is neurodevelopmental illness characterized by inattention, hyperactivity, and impulsivity due to lack of neurotransmitter dopamine in the brain [184]. Treatments for ADHD involve stimulant medications, but they may have unpleasant side outcomes. Some people utilize cannabis as ADHD therapy as cannabis restores brain dopamine levels without any adverse effects [185].

Moreover, clinical and anecdotal data recommend cannabis as a therapy for ADHD. In a study of 268 separate online discussion threads, 25% of people thought that cannabis was helpful [186]. However, efficiency of cannabis was not entirely established [187]. Another controlled clinical trial investigated effect of Sativex (THC:CBD, 1:1 ratio) on 30 ADHD patients. The study showed improvements in hyperactivity/impulsivity, inattention, and emotional lability [188]. Smoking cannabis improved the driving skills of a cannabis-user with ADHD during a time of abstinence [189]. Dronabinol produced improvement in self-injurious behaviors in adolescents [190]. High cannabis doses were related to ADHD medication reduction and a lower ADHD self-report scale score [191]. However, some research studies demonstrated that cannabis does not have impact on cognitive skills, or perhaps make the skills worse [192]. Moreover, the ability of cannabinoids to cross the placenta and affect fetal neurodevelopment may produce hyperactivity, impulsivity, and inattention symptoms in childhood [193]. Further research is needed to estimate usefulness of cannabis in the management of ADHD.

4.2 Seizure disorders

Epilepsy is one of the associated disorders in autism with an occurrence rate of 5–40% and with the highest incidence reported in adolescents and young adults [194–199]. Most studies showed increased risk of epilepsy in ASD patients, and there is also evidence indicating higher risk of ASD in individuals with epilepsy [199,200]. Careful selection of antiepileptic drugs and close monitoring of adverse effects is essential due to overlapping co-morbidities [201,202].

Cannabis was used for the treatment of epilepsy for centuries [203–205]. Cannabis, cannabinoids, and endocannabinoids have been extensively studied and found to have antiseizure activities [206–208]. In animal seizure models, AEA, THC, and WIN55212-2 exhibited potent anticonvulsant effects through CB1 activation [209,210]. But the CBD antiseizure effect was mediated by several mechanisms such as ENT transporter, GPR55, TRPV1, 5-HT1 and glycine receptors [207,211,212].

In open label study in patients with Lennox-Gastaut syndrome and drug-resistant seizures, CBD given as add-on therapy reduced seizure frequency in these highly treatment-resistant patients [213,214]. CBD also significantly reduced convulsive-seizure frequency in 120 young patients with the Dravet syndrome and drug-resistant seizures [213].

Epidiolex was the first FDA approved compound in cannabis for therapy of three treatment-resistant cases of seizures: Lennox-Gaustat Syndrome, Dravet Syndrome and Tuberous Sclerosis Complex, in patients 1 year of age and older [214]. This approval ensures the quality and effectiveness of CBD in seizure disorders.

4.3 Anxiety

Up to 40% of children with autism have at least one diagnosed anxiety syndrome [215–217]. The current anxiety treatment is cognitive-behavioral therapy that may not work for ASD children and anti-anxiety medications. Patients build a quick tolerance to these medications that causes thousands of overdose-associated deaths every year [218].

The effect of cannabis on anxiety is complex. THC and CBD produce opposing impacts on brain activity. THC is a psychoactive stimulant whereas CBD calms psychoactivity down [217]. THC relaxing effect is short-term and might develop memory deficiency and cognitive damage [219]. Moreover, THC has been associated with development of psychosis and increased anxiety [220,221]. In contrast, CBD inhibits the anxiogenic and psychotogenic effects provoked by THC and produces anxiolytic effect [222,223]. CBD has a pharmacological profile like atypical antipsychotic drugs and inhibits the FAAH enzyme increasing the levels of AEA [99,224–226]. High level of AEA was linked to reduced stress, anxiety, and depression. Other mechanisms of canabidiol anxiolytic effect may be activation of metabotropic receptors for serotonin or adenosine and interaction with TRPV1, $GABA_A$ and PPAR receptors [226–230]. CBD has demonstrated efficacy in animal models of anxiety and stress [231–234]. Preclinical data strongly suggest that CBD has a potential as therapy for generalized anxiety disorder, panic disorder, social anxiety disorder, obsessive–compulsive disorder, and post-traumatic stress disorder as well as prevention of the long-term anxiogenic effects of stress [235–237].

Clinical experimental data support preclinical findings. Cannabidiol pretreatment drastically diminished anxiety, cognitive and speech defects and improved forgetting traumatic memories in patients with social anxiety disorder [238–242]. Brain scans displayed blood flow changes in limbic and paralimbic brain zones as an evidence of anti-anxiety effect of the cannabinoid [242]. A large retrospective case series were conducted in 72 adults with CBD as add-on therapy. Anxiety scores decreased in 79.2%, 15.3% patients experienced worsening anxiety symptoms [243]. Other studies also reported CBD was efficient in reduction anxiety signs in different patient populations [244–246]. However, additional studies are required to verify long-term effectiveness and safety and establish appropriate dosing.

4.4 Sleep disorders

Sleep difficulties are the very frequent ASD-related syndromes influencing up to 80% of autistic children [247–250]. Nonpharmacological treatments for sleep problems in ASD children include general behavioral treatments, sleep hygiene and parent training [251,252]. Pharmacological therapy of autistic sleep disorders includes hypnotic medications, which cause significant adverse effects such as addiction, serious withdrawal, and complex sleep-related behaviors [253]. Melatonin may also cause sleep conditions. Individuals with ASD have abnormal melatonin metabolism, low melatonin levels and abnormalities in genes associated with melatonin production [35,254,255]. Use of melatonin in ASD has resulted in improved sleep parameters with minimal adverse effects [34,35,256,257].

Cannabis was studied for treatment of sleep disorders and was shown to have low potency sedative effect in mice [258]. In a large retrospective study with 166 patients, 79% reported improved sleep quality and considerable reduction of sleep time following cannabis use [259]. Cannabis components, THC and CBD cause different effects on sleep. Earlier studies have shown that THC produced a somnolent effect in humans [260–263]. However, in recent studies THC produced no effect on nocturnal sleep, reduced sleep expectancy as well as increased daytime sleep [264]. THC also significantly decreased duration of nighttime sleep, suggesting development of tolerance to the sedative effect [265]. CBD appeared to counteract the activity of THC by activating neurons in awaken-inducing brain zones including lateral hypothalamus and/or dorsal nuclei and increasing dopamine extracellular levels [264,266–268]. The CBD awakening properties were not inhibited by the sleep-inducing AEA [267,269]. CBD increased wakefulness during light-on period, increased sleep lights-off period and prevented sleep rebound after total sleep deficiency [265–268,270]. In a trial with 72 adults CBD upgraded sleep scores in 66.7% patients [243]. Oral administration of cannabidiol to ASD children has also shown improvement in ASD-associated sleep disorders [164]. CBD appears to optimize sleep and improve ASD-associated sleep problems and may have a beneficial value in autistic sleep conditions [271–274].

4.5 Mood disorders

A recent study discovered mood illnesses are prevalent in ASD patients. Children with ASD and ADHD were 2.7 times more expected to have mood ailments, compared with autistic patients without ADHD [275]. Autistic children can be mistakenly diagnosed as autism and bipolar disorder have some common symptoms. In many cases "mania" symptoms are also signs of autism [276]. ASD children are four-fold more likely to suffer depression [277]. Therapy of co-occurring autism and mood conditions is complex and standard pharmacotherapy may be ineffective.

The ECS has essential involvement in mood disorders. The system activity of the system may be modified by cannabinoids [278]. Both THC and CBD have potential to lessen symptoms of mood disorders. CBD produces antidepressant effects and demonstrates antipsychotic properties in depression, anxiety, and bipolar disorders [279–284]. THC in combination with CBD confirmed the antipsychotic effect [285]. However, results on effect of cannabis whole plant on mood conditions are confusing. In line with some studies that reported anxiety and increase symptoms of depression, many cannabis users describe an improvement in mood [286]. While the relationship between cannabis use and psychiatric illnesses was recorded [287,288], longitudinal research assessing the connection of cannabis with psychiatric conditions produced varied outcomes [289]. A number of studies correlated cannabis with increased risk of depression, anxiety, bipolar disorder, substance use disorders and psychosis [290–296], whereas others did not agree with this conclusion [295–297]. Understanding long-term consequences of cannabis use is especially important in managing pediatric conditions. Studies showed children using cannabis frequently have greater chance of depression, anxiety, schizophrenia, or bipolar disorder in later life [298–301]. However, not every cannabis user builds psychotic disease [188,193,302]. People with family history of a psychotic disease, or with schizotypal character, or with specific genes, might have the risk of psychotic disorder as a result of consistent cannabis use [303].

Not many studies advocate for the use of cannabis for psychiatric disorders [304]. The benefits have to be balanced against the significantly better recognized risks and the adverse consequences of cannabis for young people [305–307]. Further investigation should be conducted in patients with autism and mood disorders [283,308].

5. Cannabis genomic and biotechnology advancements

Application of genomic technologies including high-throughput sequencing has enabled significant advancements in the characterization of different plant species. Fundamental knowledge of plant gene content and genomic variation have been elucidated using biotechnology approaches. For most crops, the biotechnology tools are well developed. However, until recently the tools were under-developed for cannabis because of the prolonged ban of recreational cannabis as well as the strict regulation of hemp. Progress in cannabis has been made recently in the heterologous expression of cannabis genes, as well as in the study of the function of representative genes in model biological systems.

The heterologous expression of cannabinoids in yeast has been recently reported [309]. Key advantages of the yeast expression system for cannabinoid synthesis include ease of extraction and purification, less potential for heavy metal contamination and customization of cannabiboids. The full biogenesis of the main cannabinoids was generated in *Saccharomyces cerevisiae.* Study demonstrated the potential for a platform to produce cannabinoids, allowing for rigorous study of the compounds. Ultimately, the platform might be utilized in the development of therapies for a range of human health complications.

To characterize molecular differences between cannabis and hemp, the sequences of the respective plant genomes were analyzed [310]. Plant genomes frequently contain duplicated genes; gene amplification is a proven mechanism for increasing expression levels [311]. Overall, the sequence comparison demonstrated that there were few differences in gene median read depth (MRD) between a cannabis strain Purple Kush (PK) and the hemp cultivar Finola [310]. One exception is *AAE3*, a gene encoding an unknown function in PK. It is postulated that *AAE3* may have a role in cannabinoid biosynthesis [311]. It is believed that the large expansion of *AAE3* occurred through the insertion of pseudogenes into the PK genome. The differences in expression of the cannabinoid enzymes between PK and Finola are attributed to genetic variations that result in changes in gene expression. Recently, whole genome and transcriptome mapping of cannabis has been obtained using next generation sequencing (NGS) methods [312]. The information may be combined with proteomics and metabolomics to detect secondary cannabis metabolites. More significantly, NGS data provides the foundation for introducing genetic engineering in cannabis [313].

Because of the wide use of Cannabis as drugs, the effects of *Cannabis sativa* gene products are under investigation. The challenge is the number of different chemical components present in crude extracts. CBD has been studied in rodent models, but its effects remain incomplete. Evaluation of CBD in zebrafish showed that adult wild-type zebrafish altered behavior when exposed to 40 mg/mL of cannabidiol [314]. The locomotory ability of the zebrafish was influenced by the CBD, in contrast to the control of zebrafish exposed to water alone [314]. The CBD treated zebrafish exhibited a significant decrease of swimming performance, both in velocity as well as distance. Gene expression studies demonstrated differential gene expression, upregulation of some genes (*il 1b* and *il17a/f2*) and correspondingly down-regulation of other genes (*tgfba*, *s100a10b*, *ighm* and *cd4-1*).

While biotechnology of cannabis is still early, the availability of affordable next generation sequencing platforms together with heterologous expression systems and model biological systems, will accelerate the progress in the characterization of the cannabis genome and potential medicinal candidates.

6. Summary

Autism spectrum disorders are developmental disabilities producing substantial social, communication and behavioral disorders. No medication was created for the therapy of the main ASD symptoms. Pharmacological therapy attempts to address ASD-associated comorbidities. Many of the drugs used are off-label.

Due to its vital role in regulating emotion and social behaviors, the endocannabinoid system has been investigated for its association with ASD. Studies indicate contribution of endocannabinoid system dysfunction to ASD pathogenesis and suggest the ECS disfunction contribute to the behavioral deficits and neuroinflammation observed in autism. Cannabis and cannabinoids interact with the ECS and may improve ASD-associated social and cognitive impairments. Therefore, the ECS represents a possible goal for the development of ASD treatment and cannabis/cannabinoids may be effective as pharmacological therapy.

Although, clinical studies have shown promising results of cannabis treatment in ASD and associated disorders, there are limited data supporting clear effect of cannabis/cannabinoids in different phenotypes of ASD. More clinical investigations are needed to discover the efficacy, safety, and dosing of the therapy. This would be a significant advance in the treatment of autism and could lead to improved functioning and quality of life for the patients and their families.

Abbreviations

ASD, autism spectrum disorders; CBD, cannabidiol; THC, delta-9-tetrahydrocannabinol; CBDV, Cannabidivarin; ECS, endocannabinoid system; EC, endocannabinoid; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; AEA, anandamide; 2-AG, 2-arachidonoylglycerol; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase.

Author contributions

MB wrote chapters—Abstract, Cannabis in the treatment of autism, and Cannabis in the treatment of autism-associated disorders. HA wrote chapters—Introduction and updated Attention deficit hyperactivity disorder and Sleep disorder. PB wrote chapter—Changes in the endocannabinoid system in autism. ZL wrote—Cannabis genomic and biotechnology advancements and updated Changes in the endocannabinoid system in autism.

Ethics approval and consent to participate

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Conflict of interest

The authors declare no conflict of interest.

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