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Review

Endocannabinoid system involvement in autism spectrum disorder: An overview with potential therapeutic applications

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Abstract: Persistent deficits in social communication, restricted-repetitive patterns of behavior, interests, or activities are the core domains characterizing autism spectrum disorder (ASD). In this spectrum are grouped a heterogeneous and complex set of neurodevelopmental conditions. ASD shows pro-inflammatory events and immune system dysfunction. The endocannabinoid (EC) system is an intricate molecular network of lipid signaling pathways. The building-blocks are the arachidonic acid-derived compounds (anandamide, AEA) and 2-arachidonoyl glycerol (2-AG), their G-protein-coupled receptors (cannabinoid receptors CB1 and CB2), and their associated biosynthesizing and degradating enzymes. Recent evidence highlights a strong involvement of the EC system in the pathophysiology of some neuropsychiatric disorders and of ASD. Indeed, the EC system is able to regulate several metabolic and cellular pathways involved in autism, especially regulation of the immune system. ASD-related changes in the immune system involve alterations in monocyte and macrophage responses and pro-inflammatory cytokine up-regulation. It has been demonstrated that these processes are driven by EC system dysfunction, opening the way for targeting this system with novel drugs for ASD. Potentially, pharmacologic treatment with cannabidiol (CBD) is expected to increase endocannabinoid tone by increasing anandamide levels. Additionally, evidence from the literature indicates that CBD may alleviate many conditions co-occurring with ASD, such as seizures, gastro-intestinal problems, anxiety and depression, attention deficit, and sleep problems.

Keywords: autism spectrum disorder; endocannabinoid system; cannabidiol; inflammation; cytokine

1. Autism spectrum disorder (ASD)

Autism spectrum disorder (ASD), also called autism, is defined by the *Diagnostic and Statistical Manual for Mental Disorders, Fifth Edition (DSM-5)* and is characterized by persistent deficits in social communication interaction and restricted-repetitive patterns of behavior, interests, or activities. These symptoms begin in early childhood, and produce clinically significant developmental impairment [1]. The DSM-5 combined the previously separate subtypes of ASD listed in the Diagnostic and Statistical Manual for Mental Disorders, Fourth Edition (DSM-4). Autistic disorder, Asperger syndrome, pervasive developmental disorder-not otherwise specified (PDD-NOS), and childhood disintegrative disorder are now combined into one diagnosis of ASD. Two of the prominent clinical features of ASD are inflammation and neuro-immune system dysregulation [1]. ASD risk has been shown to be due to both genetic and environmental risk factors in approximately equal proportions [2].

2. Acetaminophen use and autism spectrum disorder (ASD)

In our 2008 paper, we were the first to show that acetaminophen but not ibuprofen use in children increases the risk for ASD [3]. As shown in that paper, acetaminophen use in children 1–18 years significantly increased the odds of autism by more than eight times. In children 1–18 years, when cases are limited to children with parent-reported regression in development, acetaminophen use significantly increased the odds of having autism by nearly 21 times. No significant increase in autism risk in these children was reported for ibuprofen use [3].

Further, we published a paper showing how warnings regarding analgesic use are correlated with cases of ASD in California [4]. As shown in the paper, in 1977 the FDA recommended a warning label for acetaminophen products which corresponded to a decrease in the number of ASD cases. In 1980 a warning for Reye Syndrome was issued in the US for the use of aspirin in children which caused a precipitous decrease in aspirin use in children. The warning led to an increase in acetaminophen use in children which corresponded to increased ASD cases in California. This can be seen in the difference in slope of the graph of ASD cases 1960–1980 versus the slope 1980–1990—the inflection point is 1980 when the use of aspirin in children was discouraged. In 1982 and again in 1986 seven random poisonings in the US occurred by tampering with the contents of acetaminophen capsules. The media warning not to use acetaminophen caused concerned parents to decrease acetaminophen use in children which corresponded to decreases in the number of children with ASD. After 1987, the increase in ASD cases continued its upward trend that began with a warning for the risk of Reye Syndrome with aspirin use in children. The aspirin/Reye Syndrome link has been questioned by many authors, most notably by Orlowski and colleagues in 2002 [5]. In this paper, they show that Reye Syndrome was already on the decline before a link to aspirin use was proposed. The replacement of aspirin use in children with acetaminophen use may have had the unintended consequence of increasing the rates of ASD.

A study of Spanish children by Avella-Garcia and colleagues showed a greater number of

autism spectrum symptoms in males which was dependent on the frequency of prenatal exposures to acetaminophen [6]. Liew and colleagues found that >20 weeks duration of acetaminophen exposure *in utero* increased the risk of ASD with hyperkinetic symptoms almost twofold in a cohort study of Danish children [7]. A meta-analysis of the correlation of prenatal use of acetaminophen and ASD was published in 2018 by Masarwa and colleagues. [8]. In their analysis, acetaminophen exposure during pregnancy significantly increased the risk of ASD by 23%. We have hypothesized that acetaminophen use would disrupt the normal functioning of the endocannabinoid (EC) system to produce ASD [9]. In a paper using data from the US National Database for Autism Research of the National Institute of Mental Health, we were able to show that acetaminophen use for fever in children was correlated with ASD [10]. In this paper we theorized that acetaminophen use in children would cause a decrease in endocannabinoid tone due to over-stimulation of the EC system, and that this decrease in endocannabinoid tone would be found in individuals with ASD.

Bertolini and colleagues have shown that acetaminophen produces analgesia by stimulating cannabinoid receptors in the brain [11]. These observations have been confirmed by Mallet and colleagues [12]. Our research has shown that acetaminophen use increases cortical endocannabinoid levels and changes behavior in a similar way to that of cannabinoids in the BTBR mouse model of ASD [13]. In this study, a test of sociability showed that acetaminophen at doses of 100 mg/kg and 400 mg/kg significantly increased dwelling near confined stranger mice by adult male BTBR mice.

3. Endocannabinoid system and autism spectrum disorder

The endocannabinoid (EC) system plays a significant role in ASD. Chakrabarti and colleagues (2015) have reviewed the EC system as it relates to autism [14] and found documentation that EC signaling plays a key role in many human health and disease conditions of the central nervous system. They state that the EC system brings together the following elements which are important for ASD treatment: 1) social reward responsiveness, 2) neuronal development, 3) circadian rhythms, and 4) symptoms of anxiety. Recently, the cannabinoid CBD has drawn interest as a potential treatment for ASD. Barchel and colleagues (2019) reported from a parental survey that CBD reduces comorbidities of ASD in a study of 53 children and young adults aged 4–22 years [15]. The ASD children enrolled in that study received CBD for a median duration of 66 days. As adverse effects, only somnolence and mild change in appetite were seen. The improved ASD comorbidities were: self-injury and rage attacks, hyperactivity symptoms, sleep problems and anxiety. Poleg and colleagues in 2019 reviewed available clinical and pre-clinical safety and efficacy data and suggested CBD as a potential treatment for core symptoms of ASD, even if they call for preclinical studies in order to investigate the effects of CBD in validated animal models of ASD-like behaviors [16].

Zamberletti and colleagues (2017) have reviewed the effects of cannabinoids in animal models of ASD [17]. Their review indicated that enhancing anandamide (AEA) signaling through inhibition of the enzyme for its degradation (FAAH) can increase sociability in animal models of ASD. They also reported that blocking the CB1 receptor may produce beneficial cognitive effects in one animal model. In 2018, Hosie and colleagues demonstrated in a mouse model that administration of the CB1 receptor agonist WIN55, 212-2 reduced aggressive behavior [18] which is

seen in ASD. Their results provide evidences on the involvement of EC system in altered synaptic activity in the basolateral amygdale of "autistic-like" mice. Melancia and colleagues (2018) were able to show that prenatal exposure to valproic acid (VPA) produced sex-specific consequences in a VPA rat model of ASD [19]. Their findings showed greater behavioral damage in male rats than in female rats prenatally exposed to VPA, and that increasing AEA signaling by inhibiting the enzyme for its degradation improved behavior in both sexes. Indeed, they demonstrated that VPA-treated female rats were less vulnerable on social communication, emotional reactivity and cognitive performance than VPA-treated male rats, which displayed selective deficits in social play behavior and stereotypies. In addition, VPA treatment was able to alter the phosphorylation status of CB1 receptors in a sex-specific, age-specific and tissue-specific manner. This finding is interesting because ASD is more common in males than in females. Kou and colleagues (2018) summarized effective pharmacological treatments of rats prenatally exposed to VPA which produced improvements in EC signaling and neuroinflammation, along with improvements in ASD-like phenotypes [20]. Interestingly, two of the compounds they reviewed were URB597 and PF3845 which inhibit the degradation of the endocannabinoid anandamide. Kerr and colleagues (2013) also showed that prenatal VPA exposure in rats is associated with alterations in the brain's EC system [21], and supported the hypothesis that EC dysfunction could underlie behavioral abnormalities of ASD. In 2016, Kerr and colleagues showed that the enzyme responsible for anandamide degradation, fatty acid amide hydrolase (FAAH), is important for regulation of social deficits in male rodents [22]. Indeed, systemic administration of the FAAH inhibitor PF3845 was able to reduce the deficit in social behavior in VPA-treated male rats, whereas VPA-treated female rats displayed no change in social behavior which suggests that anandamide may be an important regulator of social behavior [22].

In a controlled study of ASD, we were able to show that gene expression for cannabinoid receptors type 2 (CB2) but not type 1 (CB1) was up-regulated in peripheral blood mononuclear cells (PBMCs) [23] (Figure 1).

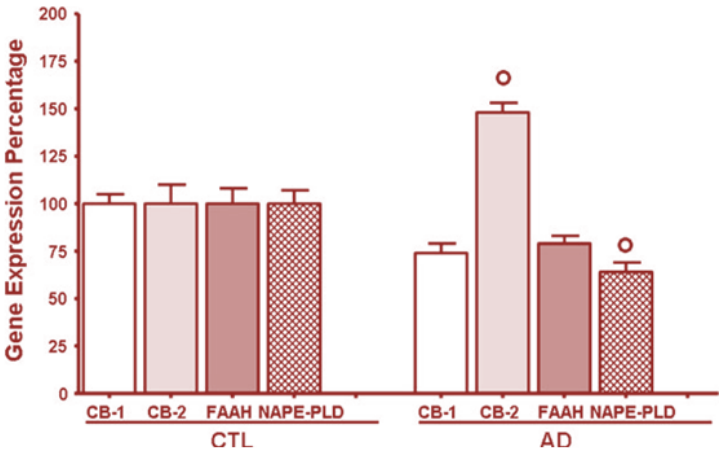

Figure 1. Gene expression changes of EC system (From reference [23] with permission of Springer).

As shown in the figure, CB2 gene expression (CB-2) was significantly higher in individuals with ASD (AD) compared to controls (CTL). In addition, the gene expression for one of the enzymes responsible for synthesis of the endocannabinoid anandamide (NAPE-PLD) is significantly lower in

individuals with ASD. This could have caused an increase in CB2 receptors resulting from a decrease in anandamide synthesis which may be indicative of decreased EC tone in ASD. Further, we have reviewed endocannabinoid signal dysregulation in ASD with emphasis on a correlation between inflammatory state and neuro-immune alterations [24]. Recently, our finding of decreased endocannabinoid tone in children with ASD was confirmed by Karhson and colleagues [25]. In this study, they were able to demonstrate that anandamide concentrations are lower in plasma of children with ASD than in control children. In 2019, Aran and colleagues also showed low circulating concentrations of cannabinoid levels in children with ASD [26]. Importantly, they also showed that, in addition to anandamide, two other FAAH substrates, palmitoylethanolamine and oleoylethanolamine, were higher in their patients treated with cannabidiol. They confirmed this result with rat brain membranes, showing that cannabidiol inhibited the action of FAAH in vitro. Our chain of thought is this: Acetaminophen use could increase the risk for ASD > Acetaminophen produces analgesia and changes in behavior through cannabinoid receptors > Over-stimulation of cannabinoid receptors decreases cannabinoid tone as seen in ASD.

4. Hypothesis: Cannabidiol could be useful to reduce immunological dysfunction in ASD and improve symptoms

Leweke and colleagues (2012) have shown that cannabidiol increases the serum levels of anandamide in schizophrenic subjects [27]. They theorized that cannabidiol inhibits the action of FAAH and thereby increases the levels of anandamide. In a survey of autism caregivers, Bar-Lev and colleagues have reported positive effects of CBD with and without the addition of tetrahydrocannabinol (THC) [28]. Although this was an epidemiologic survey and should be viewed with caution, this was the first large study to report positive CBD effects in ASD. Aran and colleagues have reported positive effects in ASD in a retrospective study of 60 children using cannabidiol-rich cannabis [26].

This leads us to our hypothesis that treating individuals with ASD by oral administration of cannabidiol will increase endocannabinoid tone by decreasing the activity of FAAH, thereby increasing plasma anandamide in individuals with ASD. The resulting increase in endocannabinoid tone should produce an amelioration of ASD symptoms. In addition, we expect that this treatment will have measureable effects on the number of CB2 receptors in PBMCs and decrease plasma measures of pro-inflammatory cytokines. We have reviewed the literature regarding the pro-inflammatory and anti-inflammatory cytokines involved ASD [29] and have summarized the cytokine profiles in blood, plasma, serum, and PBMCs that have been reported in ASD (Table 1) [29].

Decreases in pro-inflammatory cytokines, decreases in CB2 receptor gene expression, increases in anandamide levels, and improvements in standardized behavioral tests should demonstrate the progress of cannabidiol in the treatment for ASD.

Table 1. Changes in cytokine profiles in ASD.

Cytokine	Alteration	Sample
IL-1β	↑	Plasma
	↑	Serum (mRNA)
	↑	Serum
	=	Plasma
IL-2	=	Plasma
	↑	PBMC
	↓	Whole blood (mRNA)
IL-4	=	Plasma
	↑	PBMC
	=	Whole blood (mRNA)
IL-5	=	Plasma
	↑	PBMC
	↑	Plasma
IL-6	=	Plasma
	↑	Plasma
	↑	Whole blood (mRNA)
	=	Plasma
	↑	Serum
IL-8	↑	Plasma
	=	Plasma
IL-10	=	Plasma
	=	PBMC
IL-12p40	↑	Plasma
IL-12p70	↑	Plasma
IL-13	=	Plasma
	↑	PBMC
	↑	Plasma
IL-17	↑	Plasma
	↑	Serum (mRNA)
	↑	Whole blood (mRNA)
Eotaxin	↑	Plasma
S-100b	↑	Plasma
TGF-β	↑	Plasma
	=	Whole blood (mRNA)
TNF-α	=	Plasma
	↑	Serum (mRNA)
	↑	Plasma
	↑	Whole blood (mRNA)
	↑	Serum
IFN-γ	=	Plasma
	↑	PBMC

Note: Adapted from reference [29], under the Creative Commons Attribution License.

5. Cannabidiol may act through other mechanisms to treat ASD

Increasing anandamide levels with CBD treatment may provide additional mechanisms to treat ASD. The EC system has been shown to guide axons in the brain by producing a gradient for them to follow [30]. Therefore, disruption of the brain's natural endocannabinoids, such as through acetaminophen use, may interfere with proper axon positioning. Zikopoulos and colleagues have demonstrated altered anterior cingulate axon pathways in neurons of individuals with ASD which could be indicative of EC dysfunction in ASD [31]. Increasing anandamide levels has been shown to increase neurogenesis in the brain which could replace dysfunctional neurons [32]. A study by Campos and colleagues in 2013 showed that CBD increases hippocampal anandamide levels and neurogenesis in chronically stressed mice [33]. Since new neurons migrate from the hippocampus to the cortex, these new neurons following appropriate EC gradients could gradually improve symptoms of ASD. If true, this would be the first treatment that could improve the structural problems in the ASD brain.

6. Cannabidiol may alleviate conditions co-occurring with ASD

Evidence from the literature indicates that cannabidiol may alleviate many conditions co-occurring with ASD, such as seizures, gastro-intestinal problems, anxiety and depression, attention deficit, and sleep problems. The pharmacologic treatment with cannabidiol is expected to increase endocannabinoid tone by increasing anandamide levels to produce improvements in these conditions. Khan and colleagues have reported that cannabidiol exerts antiepileptic effects by restoring hippocampal interneuron functions in a temporal lobe epilepsy model [34]. Couch and colleagues and D'Argenio and colleagues have reported decreased gastrointestinal inflammation from cannabidiol use in animal models and in vitro [35,36]. Decreased depression with cannabidiol use has been reported by Sales and colleagues in animal models [37], and Lee et al. have reviewed cannabidiol's effect to reduce anxiety in animals and humans [38]. Cooper et al. have reported some evidence of decreased attention deficit by cannabinoids [39], and a beneficial effect on sleep has been reported by Babson et al. [40]. However, reports on the effect of cannabidiol on these conditions in individuals with ASD need to be explored.

7. Conclusion

Acetaminophen has been shown to produce analgesia by activation of the endocannabinoid system [11,12]. Our research has shown that acetaminophen use in children increases the risk for ASD, and have suggested that this is due to acetaminophen disruption of the endocannabinoid system [3,4,9,10]. More recent studies have also shown that prenatal exposure to acetaminophen also increases the risk for ASD [6–8]. We have suggested that acetaminophen use would decrease endocannabinoid tone which would be seen in children with ASD [9,10]. Recently, Parker and colleagues precisely reviewed the potential acetaminophen exposure and autism incidence [41]. Accordingly, the oxidative stress and inflammation seen in newborns and young children with autism could lend strength to the hypothesis of acetaminophen-induced brain injury. However, controlled clinical trials will be required in order to validate this hypothesis.

In our 2013 paper, we were able to show evidence for decreased endocannabinoid tone in children with ASD by analyzing their PBMCs. In this study, we were able to show an increase in gene expression for CB2 receptors and a decrease in gene expression for the enzyme for anandamide synthesis in these children's PBMCs [23]. Further, we have reviewed endocannabinoid signal dysregulation in ASD with emphasis on a correlation between inflammatory state and neuro-immune alterations [24]. Recently, our findings suggesting decreased endocannabinoid tone were corroborated in two studies which showed that children with ASD have decreased blood levels of the endocannabinoid anandamide and its related compounds, palmitoylethanolamide and oleoylethanolamide [25,26].

Due to the presence of low EC tone in individuals with ASD, EC system modulation could represent a possible mechanism of drug treatment for ASD. Various combinations and doses of the plant cannabinoids, cannabidiol and tetrahydrocannabinol, have been used by parents and shown to be effective in a recent survey [28], although controlled clinical trials using precise doses of cannabidiol and standardized outcome measures should be conducted to determine if there are beneficial effects of this cannabinoid and, if so, to provide recommendations for its therapeutic use.

Even if the pharmacological modulation of the EC system has been shown to be an effective treatment with in vivo and in vitro models, the agonism of the CB receptors could be linked to adverse side effects, especially related to the psychoactive chemical components of the exogenous cannabinoids, such as THC, that are required to be weighed against the clinical benefit [42]. This determination should be made by randomized placebo-controlled double blind clinical trials to demonstrate safety and tolerance. Indeed, some short- and long-term neurological adverse effects, or also paradoxical effects, are related to cannabinoid-based drugs [43]. Drug interactions with eventual concomitant medications should be also evaluated. Another point that should be further examined is the subgrouping of ASD endophenotypes. It could be possible that ASD children displaying elevated levels of inflammation could have most positive outcomes by the EC-based drugs. Small sample size of the trials performed, heterogeneity, and the complex etiology of ASD symptoms are potential limitations to develop new pharmaceutical drugs [44].

Conflict of interest

The authors declare no conflict of interest.

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