Published in final edited form as:

Epilepsy Behav. 2018 August ; 85: 237–242. doi:10.1016/j.yebeh.2018.05.016.

Proceedings of the Epilepsy Foundation's 2017 Cannabinoids in Epilepsy Therapy Workshop

Megan N. Huizenga,

Department of Pharmacology and Physiology, Georgetown University, Washington, DC, United States

Brandy E. Fureman,

Research and New Therapies, Epilepsy Foundation of America, Landover, MD, United States

Ivan Soltesz, and

Department of Neurosurgery, Stanford Neurosciences Institute, Stanford University, Stanford, CA, United States

Nephi Stella

Department of Pharmacology, Psychiatry and Behavioral Sciences University of Washington, Seattle, WA, United States

1. Introduction

The Cannabinoids in Epilepsy Therapy workshop took place at Stanford University in October 2017 and brought together researchers, clinicians, and patient advocates to facilitate an open discussion on two key topics. The first was to review the state of current research on the therapeutic value of cannabinoids (CB) and how the endocannabinoid (eCB) signaling system contributes to the control of seizures. The second was to identify where there are gaps in what we know about targeting the CB system to control seizures and disrupt seizure networks. Thus, the workshop provided opportunities for a diverse assembly of scientists, clinicians, and patient representatives to network with each other and share lessons learned about successfully researching CBs in the US. This review summarizes the key topics that were discussed and their conclusions.

Epilepsy is the 4th most prevalent neurological disorder and is characterized by spontaneous, recurrent seizures that can result in a spectrum of consequences, from disrupted quality of life, to developmental delays, injury, or early death. Despite the availability of over 25 antiseizure drugs (ASDs), 1/3 of patients still experience pharmacoresistant seizures that are not fully controlled with current ASD therapies [1]. Therefore, the identification of new effective treatment strategies is imperative. Initial anecdotal observations from patients with epilepsy, early preclinical testing, and recent human clinical trials suggest that cannabidiol (CBD), a constituent of the cannabis plant that does not trigger euphoria, may significantly

Corresponding author: mn34@georgetown.edu.

Conflict of interest

There is no conflict of interest.

reduce seizure frequency and incidence [2–4]. However, the efficacy of CBD and other compounds targeting cannabinoid receptors is significantly different depending on preclinical seizure model, including the animal strain and age, as well as the dosage and route of administration of the compounds. Human clinical trial results also suggest that seizures respond to CBD in some individuals and not in others [2, 4]. Thus, this workshop served to discuss the current scientific understanding behind CBD and other CBs for seizure control, and to determine what questions remain about their antiseizure mechanism of action and how those unknown areas can be addressed.

2. Basic science

2.1. Cannabinoid compounds, their receptors, and signaling mechanisms

The endocannabinoid (eCB) signaling system – the body's own collection of cannabinoid ligands and receptors – forms an intricate network comprised of two primary G protein-coupled receptors (GPCRs): CB₁ and CB₂ receptors (CB₁R and CB₂R), their endogenous ligands anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and the enzymes involved in their synthesis and degradation [5]. Importantly, CB₁R is the most widely expressed and abundant GPCR in the central nervous system, CB₁R is the most widely expressed on GABAergic presynaptic boutons in the cortex and hippocampus [6, 7]. On the other hand, CB₂R is predominantly expressed by hematopoietic cells and to a lesser extent by select neurons [8]. Thus, the major role of the eCB signaling system is to modulate an excitatory and inhibitory balance in the brain by distinctively controlling GABA and glutamate release, as well as by modulating the production of immunomodulators [5, 7, 8]. In fact, unlike most neurotransmitters that are synthesized and stored in presynaptic vesicles for subsequent release, 2-AG and anandamide are synthesized in an on-demand, activity-dependent manner from lipid membrane precursors [9]. This implicates the eCB signaling system as a homeostatic mechanism for regulating neuronal network activity in a state-dependent manner with a high degree of spatial and temporal selectivity. Accordingly, the functional role of the eCB signaling system in the control of the excitatory and inhibitory balance in the brain makes it an ideal target for seizure control by protecting against hyperexcitability only when and where it may occur [10].

2.2. Cannabinoids, eCB signaling, and epilepsy

Just as eCBs are produced in response to synaptic activity, evidence suggests that pathophysiological conditions, such as epilepsy, modify the eCB signaling system [10, 11]. For example, in animal models of seizures, CB₁R is downregulated acutely following seizure induction and subsequently upregulated in the chronic phase [10]. In patients with temporal lobe epilepsy, CB₁R expression in the hippocampus is down-regulated from glutamatergic axon terminals and upregulated on GABAergic terminals [10]. Therefore, the specific change in CB₁R expression by neuron subtype is thought to contribute to the mechanism underlying the development of seizures in particular neuronal networks.

Accordingly, ample evidence shows that targeting CB₁R controls seizure incidence and severity [12, 13]. In brief, activation of CB₁R with either the synthetic cannabinoid agonists, WIN55,212–2 and arachidonyl-2'-chloroethylamide (ACEA), or the phytocannabinoid, ⁹-

tetrahydrocannabinol (THC), produces pronounced antiseizure effects in multiple preclinical seizure models with subtle differences between seizure type and induction method, the dose of the compound, and animal age [13]. By contrast, CB₁R antagonists elicit proconvulsant effects across most, if not all, preclinical models [13]. This may not be unexpected because most cannabinoid agonists are quite multimodal compared with cannabinoid antagonists, often acting at both CB₁R/CB₂R and transient receptor potential (TRP) channels [14, 15]. More importantly, the potent activity of THC at CB₁R mediates its euphoric effects and limits its therapeutic potential [15].

By sharp contrast, CBD does not produce euphoric effects perhaps partly because of its different mechanism of action. The mechanism of action of CBD is polymodal and includes: minimal potency at the orthosteric binding sites of CB₁/CB₂Rs, allosteric modulation of CB₁R, antagonism of GPR55, and activation of the transient receptor potential vanilloid (TRPV) receptor family [16]. Furthermore, unlike THC, CBD reduces seizure burden in all but one of the preclinical models tested thus far [13]. Interestingly, CBD reduces seizure frequency in most, but not all, patients with Dravet syndrome and other treatment-resistant epilepsies [2, 4]. The significant efficacy of CBD treatment shown in recent human clinical trials emphasize the need to continue preclinical testing to better understand the underlying molecular mechanisms mediating this therapeutic benefit and how to optimize it. For example, in a genetically defined mouse model of Dravet syndrome that phenocopies the human condition, CBD not only reduces seizure frequency as observed in the recent human clinical trial, but also reduces seizure duration and severity [17]. It should be emphasized that acute CBD treatment also provided significant benefit for the behavioral comorbidities associated with the disease by rescuing impaired social behaviors that mirror autism-like deficits [17]. Finally, mechanistic studies show that these therapeutic effects correlate with increased GABAergic signaling via antagonism of GPR55 and decreased firing of excitatory neurons [17]. This work illustrates how continued preclinical evaluation of the mechanism of action of CBD will solidify and extend the scope of CBD treatment indications by providing critical molecular insights into the mechanisms mediating therapeutic benefits.

Recent evidence suggests that newly developed medicines designed to modulate eCB signaling may also be used as a distinct therapeutic modality to treat seizures. Levels of the endogenous ligands, AEA and 2-AG, are increased acutely after seizures in rodent models [18, 19]. The activity-dependent synthesis of eCBs and their agonism at cannabinoid receptors suggests that this increase acts as a negative feedback mechanism to inhibit glutamate release and dampen hyperexcitatory circuits [10, 20]. In line with this view, increases in eCB levels triggered by small molecule inhibitors of eCB reuptake and hydrolysis also yield primarily antiseizure effects [13]. A clear advantage linked to the inhibition of select eCB-hydrolyzing enzymes is to selectively control eCB signaling where these endogenous ligands are produced by highly active neurons without broadly activating the CB₁R throughout the brain and producing euphoric and cognitive impairing effects [21]. A search for novel eCB hydrolyzing enzymes has identified the serine hydrolase, ABHD6, as a factor controlling the level of 2-AG at CB receptors [21]. In fact, ABHD6 inhibition decreases pentylenetetrazole (PTZ)-induced seizure incidence and severity, an effect that was sustained in CB₁R and CB₂R knockout mice [22]. These remarkable CB receptor-independent effects were blocked by the application of the GABA_A antagonist picrotoxin,

which agrees with reports showing that 2-AG exerts a positive allosteric modulatory effect on the GABA_A receptor. This mechanism associated with ABHD6 inhibition is advantageous in that it may preclude chronic treatment-induced tolerance associated with targeting the orthostatic binding site of GPCRs [22, 23]. Collectively, this evidence suggests that ABHD6 inhibition could be a novel therapeutic strategy for epilepsy, although requiring further characterization of responsive seizure types.

It is important to note that the strategy of modulating eCB signaling through the inhibition of eCB inactivating enzymes for therapeutic benefit is complicated by significant interactions with presynaptic proteins connecting neurons at the synapse, such as neurexins. Indeed, an additional layer of regulation recently discovered for eCBs comes from presynaptic β -neurexins which might contribute to the modulation of postsynaptic eCB synthesis via a transsynaptic regulatory loop that alters tonic postsynaptic synthesis [24, 25]. Thus, selective targeting of eCB signaling may come from a more in-depth molecular understanding of the steady state of eCB signaling as an intrinsic and constitutive activator of CB₁R signaling that will then be adjusted by other cell signaling events [26]. Together, the work that was dedicated to better understand CB, eCB signaling, and epilepsy led to discovering how the signaling and function of the eCB system under physiological and pathophysiological conditions are changed, and provided a better understanding of how to target this signaling network with novel candidate compounds that are likely to move into more translational studies.

3. Translational

3.1. The work of the Epilepsy Therapy Screening Program on CBs

The Epilepsy Therapy Screening Program (ETSP) [27] has been instrumental in the identification and evaluation of new potential drug therapies for the treatment of epilepsy. As mentioned above, in spite of a wealth of preclinical data and the availability of over 25 ASDs, 30% of patients continue to experience seizures not fully controlled with current ASDs [1]. To address this unmet need, the ETSP refined its drug screening approach to focus on preclinical models characterized by pharmacoresistant seizures. The ETSP prioritized two acute seizure models: the maximal electroshock and 6-Hz 44-mA stimulation tests that represent generalized tonic-clonic seizures and focal seizures, respectively [28, 29]. While these models exhibit electrographic signatures similar to that of human patients with epilepsy, both occur in neurologically intact animals devoid of the neuronal network remodeling resulting in behavioral alterations that are common in human patients with epilepsy. Therefore, corneal kindling and spontaneous bursting hippocampal slice models were included to identify compounds that may only be effective in a hyperexcitable network and are resistant to many traditional ASDs [28]. Additionally, screening in the Theiler's murine encephalomyelitis virus (TMEV) model of viral encephalitis-acquired epilepsy is characterized by handling induced seizures, hippocampal sclerosis, and neuroinflammation. This model is currently employed to help identify ASDs with potential disease-modifying properties [28].

Based on the preclinical evidence that CBD exhibits antiseizure effects, the ETSP investigated in greater detail the translational potential of CBD as an antiseizure drug by

subjecting this compound to their refocused screening paradigm. In the acute seizure models, CBD displayed dose-dependent seizure protection at doses that did not impair motor function [30]. Similarly, CBD exerts dose-dependent protection against the chronic corneal kindling model, suggesting that CBD treatments may suppress seizure spread and focal activity [30]. Importantly, in the lamotrigine-resistant amygdala kindling model, where evoked seizures are resistant to lamotrigine and other select ASDs, CBD did not modify behavioral or electrographic seizure events, indicating that this compound does not have broad nonspecific effects on seizures and instead reduces seizures in a subset of models [30, 31]. Preliminary evidence suggests that CBD produces antiseizure effects in the TMEV model of viral encephalitis-induced epilepsy, as indicated by its ability to reduce the total number of seizures and cumulative seizure burden without halting hippocampal atrophy [32]. This work suggests that CBD may be preferentially efficacious against generalized tonic-clonic seizures than against clonic seizures [30]. While broadly exerting antiseizure effects in most models, the potency of CBD differs among the models tested. A possible explanation of this difference is that the therapeutic response of CBD could be due to its action at distinct targets depending on the model. This work further emphasizes the need for a better understanding of CBD's effects on its several molecular target candidates in each epileptic conditions, as well as if and how its therapeutic effect is affected when used in combination with additional ASDs and other drug therapies, as recently reviewed [33].

The ETSP analyzed combinatorial effects of CBD and other ASDs using drug combination analysis with isobolograms to determine drug synergy in preclinical models of motor seizures. Preliminary data suggest a synergistic relationship between CBD and levetiracetam in a 1-to-1 ratio [34]. Although preliminary, this finding is significant considering that CBD is in clinical trials for pediatric refractory epilepsies and levetiracetam is approved for use in children as young as 1 month of age. Furthermore, a synergistic effect may provide an opportunity for combined therapy approach with increased safety and efficacy by lowering doses of each agent. In fact, in the TMEV model, 180 mg/kg was the only dose of CBD that reduced seizure burden [34], and yet in combination with levetiracetam, 50 mg/kg of CBD showed significant efficacy [34].

Antagonistic interactions have been initially suggested between carbamazepine (1:3 and 3:1 ratio) and clobazam (1:3 ratio) when used in combination with CBD [34]. These findings are based on single dose modeling, and thus, chronic modeling remains to be established. As most patients are engaged in polytherapy, these initial findings provide an initial and essential safety results for the use of CBD with concurrent ASDs. Moving forward, this work should focus on increasing the number of drug combination studies, subchronic and chronic dosing paradigms, and an analysis of benefit on the cognitive and behavioral comorbidities associated with epilepsy.

Technological advancements to help better understand the antiseizure mechanism of CBs

Several cutting-edge technologies are being developed to refine and enhance the real-time visualization of neuronal network dynamics providing a unique opportunity for their application in preclinical seizure models. First, the development of optogenetic and chemogenetic techniques have provided enhanced temporal and spatial control over specific

neuronal populations, allowing for functional circuit mapping [35]. Individually, both techniques offer potential for future clinical treatment strategies similar to what was achieved for deep brain stimulation approaches [36, 37]. Additional technological advancements have allowed for enhanced large-scale imaging of the brain at the cellular level, and often in real time. The use of genetically encoded and virally delivered indicators of neural Ca^{2+} dynamics, for example, provides a powerful imaging modality that enables the analysis of large-scale neural activity data. Two such approaches allow for optical brain imaging in behaving animals that are either head-fixed or freely moving. The head-fixed paradigm utilizes conventionally sized optical instruments, often a high performance two-photon microscope, allowing activity in fine structures to be detected [38]. Furthermore, one can now overcome the limitation of investigating neural dynamics associated with behaviors that are incompatible with head restraint by using miniaturized and head-mounted microscopes that allow for brain imaging in freely behaving animals. Thus, cellular level imaging techniques used in combination with optogenetic approaches enable simultaneous neural imaging and manipulation of spiking dynamics during awake animal behavior [38]. These advancements have direct impact on epilepsy research by allowing both the visualization and manipulation of seizure networks in freely moving animal models of epilepsy and how this is impacted by ASDs.

An additional promising technology is a preparation termed “Crystal Skull”, which was created to provide long-term optical access to the cortex in behaving mice by replacing the dorsal cranium with curved window glass [39]. This technique builds on previous technological advancements and provides broader regional access to the mammalian brain and should allow for tracking the cellular dynamics of millions of cells in the awake, behaving mouse. Similar to the miniaturized head-mounted microscopes, implementing the Crystal Skull technique in transgenic mice expressing fluorescent Ca^{2+} indicators offers measurement of neuronal discharges through Ca^{2+} activity in millions of cortical neurons via two-photon imaging [39]. This technique is an exciting advancement in the epilepsy field given the potential for chronic monitoring of multiple brain areas and quantification of dynamic neuronal signaling in epileptically vulnerable networks and how this is impacted by ASDs.

Nanoscale super-resolution imaging constitutes an additional advancement that allows for the characterization of discrete physiological features of individual receptors and signaling patterns. For example, stochastic optical reconstruction microscopy (STORM) now enables characterization of the cell type specific localization and relative density of CB_1R via super-resolution imaging [40]. The ability to precisely map the subcellular location of CB_1R at the synaptic boutons is essential to better understand its involvement in epilepsy pathogenesis, where CB_1Rs are often downregulated on excitatory neurons in patients with temporal lobe epilepsy. While the STORM technique in itself does not readily allow for cell type identification, it can be overlaid on individual neurons labeled with biocytin. Subsequent histochemical staining against synaptic elements, such as the presynaptic scaffolding protein Bassoon allows the visualization of synaptic structure [40]. Furthermore, in combination with patch-clamp electrophysiology and confocal imaging of biocytin-filled neurons overlaid with STORM, super-resolution imaging enables quantitative physiological and morphological data collection from the same neuron [40, 41]. This technique revealed that

THC causes robust internalization of CB₁R only at high doses. For example, following 6 days of 10 mg/kg THC treatment in mice, 74% of CB₁Rs are lost from axon terminals of inhibitory GABAergic interneurons, with full recovery of receptor density occurring 6 weeks after drug cessation [41]. These results suggest that chronic THC administration underlies the decreased efficacy of CB effects on GABAergic currents by directly reducing the number of CB₁Rs from axon terminals. Of note, many medical cannabis preparations are designed to limit the euphoria and cognitive impairment effects by reducing THC levels and accordingly, medical cannabis preparations with low THC and high CBD concentrations are often sought out as alternative epilepsy treatments [42]. In a similar experiment, a lower (1 mg/kg) dose of THC that does not provoke behavioral effects on rodents only decreases CB₁R density by 16% without any receptor internalization [40]. Therefore, STORM imaging revealed that THC administration results in a dose-dependent decrease in CB₁R from GABAergic axon terminals which likely contributes to persistent alterations in synaptic transmission. The STORM imaging technique successfully permits the study of cell specific molecular distribution and changes of proteins such as CB₁R at the nanoscale level. Together, the advancements that have been made in both preclinical compound screening and technologies that allow for the real-time monitoring of neuronal functions should help the field better understand the molecular, cellular, and systems mechanisms by which CBs might provide therapeutic benefit for pharmacoresistant epilepsy.

4. Clinical

Interest in cannabis-based treatment options for epilepsy has greatly increased following media coverage of anecdotal reports and increased access to medical cannabis products. Accordingly, parents of children with treatment-resistant seizures are turning to alternative treatment options, namely cannabidiol-enriched cannabis products. In fact, in a retrospective chart review of children and adolescents who used oral cannabis extracts as an epilepsy treatment, 41% relocated to Colorado, where retail sale of cannabis is legal [43]. Furthermore, the Colorado Medical Marijuana Registry Program reports that seizures are the most commonly cited indication for cannabis use. Although parents report at least some improvement in seizures following the use of cannabis products, their use may also pose significant risk to children and young adults. Preclinically, prolonged exposure of the embryo in utero to exogenous THC or the synthetic CB₁R agonist WIN55,212-2 results in a loss of CB₁R prominently in cholecystokinin-expressing interneurons (CCK-INTs). This loss is associated with persistent deficits in inhibitory circuitry and impairments in social behavior [44].

The preclinical evidence of the detrimental effect of prolonged cannabinoid exposure on neuronal function might provide an explanation to several reports in humans. For example, cannabis use by young adults is correlated with detrimental effects on the developing brain [45–47]. Therefore, a greater understanding of cannabis-based products is needed to determine their effect on the brain. However, a lack of regulation and standardization in the manufacturing of medical cannabis products creates significant variability in the products used [42]. A factor that contributes to product variability is a limited understanding of cannabis plant biology and genomic strain variation. For example, a misleading perception exists that the two major species of the cannabis plant impart distinct psychological effects.

The most common one is that the *Cannabis indica* species, a short and wide cannabis plant, leads to more sedating effects, while the *Cannabis sativa* species, a long and tall cannabis plant, triggers more uplifting effects [48]. Current research is showing that the cannabis plant morphology and THC:CBD ratio is tightly regulated by intricate genetic mechanisms that can be controlled by breeding defined strains [48]. Thus, plant morphology alone cannot provide information regarding the relative cannabinoid composition [48]. For example, an assessment of the same cannabis sample advertised to have low THC, high CBD levels from five dispensaries in the Seattle area, CBD content ranged from 0% to 75% CBD [48]. It is important to emphasize here that despite product inconsistency, the use of artisanal cannabis preparations continues to increase because effective treatments for highly intractable epilepsies remain scarce or even unavailable. This research highlights the urgent need to gather safety and efficacy results on CBD and cannabinoid combination therapies for treatment-resistant epilepsies.

Clinical reports of the anticonvulsant properties of CBD in humans are not new as 4 controlled studies were conducted between 1978 and 1990 examining the effect of CBD on seizures [15, 49–51]. While two of the 4 studies reported small effects of CBD on seizure frequency, all of the studies had noticeable limitations, including small sample size, incomplete data collection, and instances of insufficient blinding. Two recent trials addressed the potential therapeutic benefit of CBD for treatment-resistant epilepsies. The first was an open-label intervention trial that determined if the addition of CBD to the existing ASD treatment regimens is safe, tolerable, and effective in children and young adults with highly intractable epilepsies. Adverse events were reported in 79% of participants, with serious adverse events related to the CBD treatment accounting for 12%, half of which were status epilepticus [2]. Specifically, there was significant drug interaction between CBD and clobazam likely due to the inhibition of the enzyme CYP2C19 by CBD leading to increased concentrations of an active metabolite of clobazam (*N*-desmethyl clobazam) by as much as 60% in some participants [2, 52]. This metabolite is reported to exert some anticonvulsant as well as toxic effects [53]. The primary outcome endpoint was the percent change in motor seizure frequency per month. The median monthly motor seizure frequency decreased from 30 during the baseline period to 15.8 over the 12-week CBD treatment period [2].

After completion of the trial, all participants were able to continue treatment through a long-term, open-label study. Preliminary findings from the open-label study revealed that over a three-year treatment period with CBD, therapeutic benefit is likely to be primarily due to the effects of CBD alone. Specifically, out of the 25 participants, only 3 reported a lack of effect with CBD treatment, and 4 did not experience enhanced seizure reduction with a THC combination therapy compared with CBD alone [54]. As with most drug treatments, researchers found that with CBD, participants either responded very well, minimally, or had no or adverse responses. In addition, most participants experienced significant cognitive gains while using CBD. In the separate group of participants with tuberous sclerosis complex (TSC), a genetic disease resulting in the growth of tumors with over 90% of affected patients developing intractable epilepsy, all 18 participants had improved cognition [54]. This finding is particularly striking because TSC is associated with significant developmental delays, learning difficulties, and autism, and all participants treated with

CBD improved cognitively regardless of seizure response. In fact, half of the participants' seizures did not respond to CBD treatment, but all those individuals still exhibited cognitive gains. Therefore, while cognitive improvements were reported in the initial trial, these data highlight the possibility that the use of CBD alone may, in fact, trigger changes in select cognitive responses, in addition to seizure reduction.

Results from the open-label trial suggest that CBD has an acceptable safety profile and reduces the frequency of motor seizures in children and young adults with treatment-resistant epilepsy [54]. However, study limitations were acknowledged and included variability in patient eligibility criteria between study sites [54] and potential bias of parental-reported effects and drug–drug interactions that may have led to an overestimation of CBD efficacy [2, 54], which warranted further characterization of the safety and efficacy of CBD through more rigorous randomized controlled trials.

A double-blind, placebo-controlled trial was conducted to assess the efficacy of CBD against the drug-resistant seizures that characterize Dravet syndrome. Dravet syndrome is a rare devastating genetic form of epileptic encephalopathy with highly drug-resistant seizures and high mortality rate during young adulthood. In the earlier open-label trial, participants with Dravet syndrome comprised the largest cohort, with those participants experiencing a median reduction in monthly motor seizures of 49.8%, supporting the need for further investigation [2]. Similar to the open label trial, the primary outcome measure in the double-blind, placebo-controlled Dravet trial was median frequency in monthly convulsive seizures, which were decreased from 12.4 to 5.9 with CBD, compared with a decrease from 14.9 to 14.1 with placebo [4]. Furthermore, 43% of participants in the CBD arm experienced at least a 50% seizure reduction, compared with only 27% reaching at least a 50% seizure reduction in the add-on placebo group, without affecting the number of nonconvulsive seizures [4]. However, adverse events were more prevalent in the CBD group, with 93% of patients reporting adverse events compared with 75% of patients in the placebo group [4]. Together, these data showed that adjunct CBD treatment provides a significant reduction in convulsive seizure frequency among children and adolescents with Dravet syndrome, but was also associated with select adverse events compared with placebo, including gastrointestinal effects, fatigue, decreased appetite, and somnolence. While these are very exciting results for a population with highly refractory seizures, these clinical trials reinforce the need for additional data on the long-term effects and efficacy of cannabinoid-based treatments for seizure control in this and other refractory epilepsy populations, with the goal of optimizing this novel therapeutic modality.

5. Future directions

In summary, the workshop ascertained the current state of cannabinoid-based therapeutics for the treatment of epilepsy and helped identify several key knowledge gaps that still remain to be addressed.

First, one of the fundamental issues that persist in epilepsy research is determining the preclinical animal models to use that best recapitulate each human condition. Suggestions include the use of genetic models as well as adopting a pluripotent stem cell approach from

patients with genetic mutation and/or highly refractory seizures. Second, the initial reports of success in CBD use for refractory epilepsy call for an increased understanding of its mechanism of action with the goal of optimizing this response. This should include CBD's interaction with select targets and assessment of signaling patterns under normal and pathophysiological conditions, as well as CBD metabolism, and testing its metabolites for bioactivity. Moreover, while initial evidence suggested that CBD appears to act through CB1R-independent mechanisms, its activity as an allosteric modulator of the CB1R is a signaling mechanism warranting further investigation. Along those lines, the effect of CBs on glial cells also warrants investigation, specifically when considering the possible involvement of CB actions on CB₂R and possible roles in modulating neuroinflammation triggered by epilepsy.

It is our hope that this workshop has served to enhance the crosstalk between researchers, clinicians, and patient representatives to help identify key research areas that should be explored in the near future to provide the most targeted and effective cannabinoid-based therapeutic options for the treatment of epilepsy.

Acknowledgements

We gratefully acknowledge the expert contributions of the workshop speakers, including Angela Birnbaum, PhD (University of Minnesota), Maja Djuristic PhD (Stanford University), Barna Dudok, PhD (Stanford University), Ariel Flores (Department of Justice), Keith Humphreys, PhD (Stanford University), Catherine Jacobson, PhD (Patient Panelist), Kelly Knupp, MD (Children's Hospital Colorado), Ken Mackie, MD (Indiana University), Chris McBain, PhD (National Institutes of Health), Evelyn Nussenbaum (Patient Panelist), Brenda Porter, MD, PhD (Stanford University), Mark Schnitzer, PhD (Stanford University), Misty Smith, PhD (University of Utah), Ivan Soltesz, PhD (Stanford University), Nephi Stella, PhD (University of Washington), Thomas Sudof, MD, PhD (Stanford University), Elizabeth Thiele, MD, PhD (Massachusetts General Hospital/Harvard University), Jessica Tonani, CEO (Verda Bio), Ben Whalley, PhD (GW Pharmaceuticals), and Karen Wilcox, PhD (University of Utah).

Funding

This workshop was supported by the Epilepsy Foundation, the Tuberous Sclerosis Alliance, and an unrestricted educational grant from Greenwich Biosciences.

References

- [1]. Kwan P, Brodie MJ. Early identification of refractory epilepsy. *N Engl J Med* 2000;342 (5):314–9. 10.1056/NEJM200002033420503. [PubMed: 10660394]
- [2]. Devinsky O, Marsh E, Friedman D, Thiele E, Laux L, Sullivan J, et al. Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. *Lancet Neurol* 2016;15(3): 270–8. 10.1016/S1474-4422(15)00379-8. [PubMed: 26724101]
- [3]. Friedman D, Devinsky O. Cannabinoids in the treatment of epilepsy. *N Engl J Med* 2015;373(11): 1048–58. 10.1056/NEJMra1407304. [PubMed: 26352816]
- [4]. Devinsky O, Cross JH, Laux L, Marsh E, Miller I, Nabbout R, et al. Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. *N Engl J Med* 2017;376(21): 2011–20. 10.1056/NEJMoa1611618. [PubMed: 28538134]
- [5]. Di Marzo V. Endocannabinoids: synthesis and degradation. In: Amara SG, Bamberg E, Fleischmann B, et al., editors. *Reviews of physiology, biochemistry and pharmacology* Berlin Heidelberg: Springer; 2006 p. 1–24. 10.1007/112_0505.
- [6]. Marsicano G, Lutz B. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. *Eur J Neurosci* 1999;11(12):4213–25. 10.1046/j.1460-9568.1999.00847.x. [PubMed: 10594647]

- [7]. Katona I, Sperlagh B, Sikló, Kálfalvi A, Vizi ES, Mackie K, et al. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci* 1999;19(11):4544–58. [PubMed: 10341254]
- [8]. Cabral GA, Griffin-Thomas L. Emerging role of the CB2 cannabinoid receptor in immune regulation and therapeutic prospects. *Expert Rev Mol Med* 2009;11:e3 10.1017/S1462399409000957. [PubMed: 19152719]
- [9]. Stella N, Piomelli D. Receptor-dependent formation of endogenous cannabinoids in cortical neurons. *Eur J Pharmacol* 2001;425(3):189–96. 10.1016/S0014-2999(01)01182-7. [PubMed: 11513837]
- [10]. Soltesz I, Alger BE, Kano M, Lee SH, Lovinger DM, Ohno-Shosaku T, et al. Weeding out bad waves: towards selective cannabinoid circuit control in epilepsy. *Nat Rev Neurosci* 2015;16(5): 264 10.1038/nrn3937. [PubMed: 25891509]
- [11]. Hofmann ME, Frazier CJ. Marijuana, endocannabinoids, and epilepsy: potential and challenges for improved therapeutic intervention. *Exp Neurol* 2013;244:43–50. 10.1016/j.expneurol.2011.11.047. [PubMed: 22178327]
- [12]. Rosenberg EC, Tsien RW, Whalley BJ, Devinsky O. Cannabinoids and epilepsy. *Neurotherapeutics* 2015;12(4):747–68. 10.1007/s13311-015-0375-5. [PubMed: 26282273]
- [13]. Rosenberg EC, Patra PH, Whalley BJ. Therapeutic effects of cannabinoids in animal models of seizures, epilepsy, epileptogenesis, and epilepsy-related neuroprotection. *Epilepsy Behav* 2017;70(Pt B):319–27. 10.1016/j.yebeh.2016.11.006. [PubMed: 28190698]
- [14]. Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ^9 -tetrahydrocannabinol, cannabidiol and Δ^9 -tetrahydrocannabivarin. *Br J Pharmacol* 2008;153(2): 199–215. 10.1038/sj.bjp.0707442. [PubMed: 17828291]
- [15]. Devinsky O, Cilio MR, Cross H, Fernandez-Ruiz J, French J, Hill C, et al. Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. *Epilepsia* 2014;55(6):791–802. 10.1111/epi.12631. [PubMed: 24854329]
- [16]. Ibeas Bih C, Chen T, Nunn AVW, Bazet M, Dallas M, Whalley BJ. Molecular targets of cannabidiol in neurological disorders. *Neurotherapeutics* 2015;12(4):699–730. 10.1007/s13311-015-0377-3. [PubMed: 26264914]
- [17]. Kaplan JS, Stella N, Catterall WA, Westenbroek RE. Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. *Proc Natl Acad Sci* 2017; 114(42):11229–34. 10.1073/pnas.1711351114. [PubMed: 28973916]
- [18]. Wallace MJ, Blair RE, Falenski KW, Martin BR, Delorenzo RJ. The endogenous cannabinoid system regulates seizure frequency and duration in a model of temporal lobe epilepsy. *J Pharmacol Exp Ther* 2003;307(1):129–37. 10.1124/jpet.103.051920. [PubMed: 12954810]
- [19]. Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, et al. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* 2003;302(5642): 84–8. 10.1126/science.1088208. [PubMed: 14526074]
- [20]. Katona I, Freund TF. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nat Med* 2008;14(9):923 10.1038/nm.f.1869. [PubMed: 18776886]
- [21]. Marrs WR, Blankman JL, Horne EA, Thomazeau A, Lin YH, Coy J, et al. The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. *Nat Neurosci* 2010;13(8):951 10.1038/nn.2601. [PubMed: 20657592]
- [22]. Naydenov AV, Horne EA, Cheah CS, Swinney K, Hsu KL, Cao JK, et al. ABHD6 blockade exerts antiepileptic activity in PTZ-induced seizures and in spontaneous seizures in R6/2 mice. *Neuron* 2014;83(2):361–71. 10.1016/j.neuron.2014.06.030. [PubMed: 25033180]
- [23]. Sigel E, Baur R, Rácz I, Marazzi J, Smart TG, Zimmer A, et al. The major central endocannabinoid directly acts at GABAA receptors. *Proc Natl Acad Sci U S A* 2011; 108(44): 18150–5. 10.1073/pnas.1113444108. [PubMed: 22025726]
- [24]. Anderson GR, Aoto J, Tabuchi K, Földy C, Covy J, Yee AX, et al. β -Neurexins control neural circuits by regulating synaptic endocannabinoid signaling. *Cell* 2015;162(3):593–606. 10.1016/j.cell.2015.06.056. [PubMed: 26213384]
- [25]. Südhof TC. Synaptic neurexin complexes: a molecular code for the logic of neural circuits. *Cell* 2017;171(4):745–69. 10.1016/j.cell.2017.10.024. [PubMed: 29100073]

- [26]. Südhof TC. Tonic CB1 signaling. Presented at the: Cannabinoid Signaling in Epilepsy: Pathways to Therapy workshop Stanford University; 2017.
- [27]. Epilepsy Therapy Screening Program (ETSP), National Institute of Neurological Disorders and Stroke Available from: <https://www.ninds.nih.gov/current-research/focus-research/focus-epilepsy/etsp>, Accessed date: 14 March 2018.
- [28]. Barker-Haliski ML, Johnson K, Billingsley P, Huff J, Handy LJ, Khaleel R, et al. Validation of a preclinical drug screening platform for pharmacoresistant epilepsy. *Neurochem Res* 2017;42(7): 1904–18. 10.1007/s11064-017-2227-7. [PubMed: 28303498]
- [29]. Barton ME, Klein BD, Wolf HH, Steve White H. Pharmacological characterization of the 6 Hz psychomotor seizure model of partial epilepsy. *Epilepsy Res* 2001;47(3): 217–27. 10.1016/S0920-1211(01)00302-3. [PubMed: 11738929]
- [30]. Klein BD, Jacobson CA, Metcalf CS, Smith MD, Wilcox KS, Hampson AJ, et al. Evaluation of cannabidiol in animal seizure models by the Epilepsy Therapy Screening Program (ETSP). *Neurochem Res* 2017;42(7):1939–48. 10.1007/s11064-017-2287-8. [PubMed: 28478594]
- [31]. Postma T, Krupp E, Li X-L, Post RM, Weiss SRB. Lamotrigine treatment during amygdala-kindled seizure development fails to inhibit seizures and diminishes subsequent anticonvulsant efficacy. *Epilepsia* 2000;41(12):1514–21. 10.1111/j.1499-1654.2000.001514.x. [PubMed: 11114208]
- [32]. Wilcox KS. NINDS Epilepsy Therapy Screening Program for CB-targeted compounds. Presented at the: Cannabinoid Signaling in Epilepsy: Pathways to Therapy workshop Stanford University; 2017.
- [33]. Patel DC, Wilcox KS, Metcalf CS. Novel targets for developing antiseizure and, potentially, antiepileptogenic drugs. *Epilepsy Curr* 2017;17(5):293–8. 10.5698/1535-7597.17.5.293. [PubMed: 29225544]
- [34]. Smith MD. Therapeutic prospective for cannabidiol: antiseizure drug-drug interactions. Presented at the: Cannabinoid Signaling in Epilepsy: Pathways to Therapy workshop Stanford University; 2017.
- [35]. Forcelli PA. Applications of optogenetic and chemogenetic methods to seizure circuits: where to go next? *J Neurosci Res* 2017;95(12):2345–56. 10.1002/jnr.24135. [PubMed: 28791729]
- [36]. Li MCH, Cook MJ. Deep brain stimulation for drug-resistant epilepsy. *Epilepsia* 2018; 59(2): 273–90. 10.1111/epi.13964. [PubMed: 29218702]
- [37]. Rossi MA, Go V, Murphy T, Fu Q, Morizio J, Yin HH. A wirelessly controlled implantable LED system for deep brain optogenetic stimulation. *Front Integr Neurosci* 2015; 9:8 10.3389/fnint.2015.00008. [PubMed: 25713516]
- [38]. Hamel EJO, Grewe BF, Parker JG, Schnitzer MJ. Cellular level brain imaging in behaving mammals: an engineering approach. *Neuron* 2015;86(1):140–59. 10.1016/j.neuron.2015.03.055. [PubMed: 25856491]
- [39]. Kim TH, Zhang Y, Lecoq J, Jung JC, Li J, Zeng H, et al. Long-term optical access to an estimated one million neurons in the live mouse cortex. *Cell Rep* 2016;17(12): 3385–94. 10.1016/j.celrep.2016.12.004. [PubMed: 28009304]
- [40]. Dudok B, Barna L, Ledri M, Szabó SI, Szabadits E, Pintér B, et al. Cell-specific STORM super-resolution imaging reveals nanoscale organization of cannabinoid signaling. *Nat Neurosci* 2015;18(1):75 10.1038/nn.3892. [PubMed: 25485758]
- [41]. Barna L, Dudok B, Miczán V, Horváth A, László ZI, Katona I. Correlated confocal and super-resolution imaging by VividSTORM. *Nat Protoc* 2016;11(1):163 10.1038/nprot.2016.002. [PubMed: 26716705]
- [42]. Porter BE, Jacobson C. Report of a parent survey of cannabidiol-enriched cannabis use in pediatric treatment-resistant epilepsy. *Epilepsy Behav* 2013;29(3):574–7. 10.1016/j.yebeh.2013.08.037. [PubMed: 24237632]
- [43]. Treat L, Chapman KE, Colborn KL, Knupp KG. Duration of use of oral cannabis extract in a cohort of pediatric epilepsy patients. *Epilepsia* 2017;58(1):123–7. 10.1111/epi.13617. [PubMed: 27859038]

- [44]. Vargish GA, Pelkey KA, Yuan X, Chittajallu R, Collins D, Fang C, et al. Persistent inhibitory circuit defects and disrupted social behavior following in utero exogenous cannabinoid exposure. *Mol Psychiatry* 2017;22(1):56–67. 10.1038/mp.2016.17. [PubMed: 26976041]
- [45]. Turkanis SA, Karler R. Central excitatory properties of delta 9-tetrahydrocannabinol and its metabolites in iron-induced epileptic rats. *Neuropharmacology* 1982;21(1):7–13. [PubMed: 6278353]
- [46]. Meier MH, Caspi A, Ambler A, Harrington H, Houts R, Keefe RS, et al. Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proc Natl Acad Sci* 2012;109(40):E2657–64. 10.1073/pnas.1206820109. [PubMed: 22927402]
- [47]. Wilkinson ST, Radhakrishnan R, D'Souza DC. Impact of cannabis use on the development of psychotic disorders. *Curr Addict Rep* 2014;1(2):115–28. 10.1007/s40429-014-0018-7. [PubMed: 25767748]
- [48]. Tonani J Verda Bio: understanding the plant biology of cannabis. Presented at the: Cannabinoid Signaling in Epilepsy: Pathways to Therapy workshop Stanford University; 2017.
- [49]. Cunha JM, Carlini EA, Pereira AE, Ramos OL, Pimentel C, Gagliardi R, et al. Chronic administration of cannabidiol to healthy volunteers and epileptic patients. *Pharmacology* 1980;21(3):175–85. [PubMed: 7413719]
- [50]. Ames FR, Cridland S. Anticonvulsant effect of cannabidiol. *S Afr Med J* 1986;69(1):14. [PubMed: 3941934]
- [51]. Gloss D, Vickrey B. Cannabinoids for epilepsy. *Cochrane Database Syst Rev* 2012;6: CD009270. 10.1002/14651858.CD009270.pub2.
- [52]. Geffrey AL, Pollack SF, Bruno PL, Thiele EA. Drug–drug interaction between clobazam and cannabidiol in children with refractory epilepsy. *Epilepsia* 2015;56(8):1246–51. 10.1111/epi.13060. [PubMed: 26114620]
- [53]. Parmeggiani A, Posar A, Sangiorgi S, Giovanardi-Rossi P. Unusual side-effects due to clobazam: a case report with genetic study of CYP2C19. *Brain Dev* 2004;26(1):63–6. 10.1016/S0387-7604(03)00074-3. [PubMed: 14729419]
- [54]. Thiele EA. Clinical perspective on anti-seizure effects of CBD: the MGH experience. Presented at the: Cannabinoid Signaling in Epilepsy: Pathways to Therapy workshop Stanford University; 2017.