HHS Public Access

Author manuscript

Neurotoxicology. Author manuscript; available in PMC 2022 January 01.

Published in final edited form as:

Neurotoxicology. 2021 January ; 82: 130–136. doi:10.1016/j.neuro.2020.12.002.

Cannabidiol reduces soman-induced lethality and seizure severity in female plasma carboxylesterase knockout mice treated with midazolam.

Erica R. Kundrick[1,+], **Brenda M. Marrero-Rosado**[1,+], **Marcio de Araujo Furtado**[2,3], **Michael Stone**[1], **Caroline R. Schultz**[1], **Lucille A. Lumley**[1,*]

[1]US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010

[2]Uniformed Services University of the Health Sciences, Bethesda, MD, 20814

[3]BioSEaD, LLC. Rockville, MD, 20850

Abstract

Cannabidiol, approved for treatment of pediatric refractory epilepsy, has anti-seizure effects in various animal seizure models. Chemical warfare nerve agents, including soman, are organophosphorus chemicals that can induce seizure and death if untreated or if treatment is delayed. Our objective was to evaluate whether cannabidiol would ameliorate soman-induced toxicity using a mouse model that similar to humans lacks plasma carboxylesterase. In the present study, adult female plasma carboxylesterase knockout (Es1−/−) mice were pre-treated with cannabidiol (20–150 mg/kg) or vehicle 1 hour prior to exposure to a seizure-inducing dose of soman and evaluated for survival and seizure activity. The muscarinic antagonist atropine sulfate and the oxime HI-6 were administered at 1 min after exposure, and the benzodiazepine midazolam was administered at 30 min after seizure onset. Cannabidiol (150 mg/kg) pre-treatment led to a robust increase in survival rate and attenuated body weight loss in soman-exposed mice treated with medical countermeasures, compared to mice pre-treated with vehicle. In addition, mice pretreated with cannabidiol (150 mg/kg) had a modest reduction in seizure severity after

*Corresponding author: Lucille A. Lumley, Ph.D., US Army Medical Research Institute of Chemical Defense, 8350 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400, lucille.a.lange.civ@mail.mil.

+These two authors contributed equally.

Credit Author Statement

E.K.: Formal Analysis, Investigation, and Writing (original draft and review & editing), Visualization. **B.M.R.:** Formal Analysis, Investigation, and Writing (original draft and review & editing), Visualization. **M.D.F**. Methodology, Formal Analysis. **M.S.:** Investigation. **C.S.:** Investigation and Data Curation. **L.L** Conceptualization, Methodology, Formal Analysis, Investigation, Resources, Writing (original draft and review & editing), Supervision, Project Administration, and Funding Acquisition.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The views expressed in this manuscript are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government.

midazolam treatment compared to vehicle-pretreated. These findings of improved outcome with cannabidiol administration in a severe seizure model of soman exposure provide additional pre-clinical support for the benefits of cannabidiol against exposure to seizure-inducing chemical agents and suggest cannabidiol may augment the anti-seizure effects of midazolam.

Keywords

chemical warfare nerve agent; status epilepticus; anti-seizure; survival; cannabinoids

1. Introduction

Cannabinoids have emerged for their therapeutic potential against a broad range of neurodegenerative and psychiatric disorders, including the treatment of refractory epilepsy for which currently available treatments are ineffective in approximately 35% of patients.[1] In particular cannabidiol (CBD), a non-psychoactive component of *Cannabis,* is increasingly being studied as a treatment for pharmacoresistant epilepsy. Epidiolex (GW Pharmaceuticals), a 99% pure CBD extract, approved by the FDA for the treatment of refractory pediatric epilepsy syndromes (Asaka et al., 2006), reduces seizure severity in patients with treatment-resistant epilepsy (Szaflarski et al., 2018). Pre-treatment with CBD also has anti-seizure effects in some but not all acute seizure animal models including audiogenic focal seizures and those induced by maximal electroshock, pentylenetetrazole, amygdala electrical kindling, and cocaine, among others (Billakota et al., 2019; Klein et al., 2017; Lazarini-Lopes et al., 2020). In electroconvulsive seizure models, including the 6 Hz 44 mA mouse and maximal electroshock mouse and rat models, CBD pre-treatment prevented seizures in a dose-dependent manner, suggesting that CBD may be effective against generalized tonic-clonic seizures (Klein et al., 2017). Cocaine-induced seizures in mice, another model that mimics generalized tonic-clonic seizures in humans, has also been shown to benefit from CBD pre-treatment by reducing seizure duration and increasing the latency to seizure onset (Gobira et al., 2015). In contrast, a model of pharmacoresistant evoked seizures, the lamotrigine-resistant amygdala kindled rat, showed no protective benefit of CBD pre-treatment on severity of behavioral seizures and electrographic after discharge duration (Klein et al., 2017).

Studies evaluating the efficacy of CBD against acute organophosphorus (OP) chemical-induced toxicity are lacking. Chemical warfare nerve agents (CWNAs), including soman, are toxic OP compounds that inhibit acetylcholinesterase (Newmark, 2019). Medical countermeasures for CWNAs include administration of the muscarinic antagonist atropine sulfate, an oxime to reactivate acetylcholinesterase, and a benzodiazepine anti-convulsant. However, when benzodiazepine treatment of CWNA-induced seizure is delayed or dose is insufficient, status epilepticus develops and seizures become refractory to benzodiazepine treatment (Niquet et al., 2020; Shih et al., 1999). Rapid seizure control is important to prevent or ameliorate the deleterious consequences of acute OP exposure.

We investigated whether CBD pre-treatment might ameliorate the toxic effects of soman in midazolam-treated female plasma carboxylesterase knockout (Es1−/−) mice. Es1−/− mice

similar to humans lack plasma carboxylesterase (Duysen et al., 2011) and have a lower median lethal dose of soman compared to wild-type (C57BL/6) mice (Duysen et al., 2011; Kundrick et al., 2020) and thus may better model human OP toxicity. Midazolam dose-dependently increases survival to soman, yet when treatment is delayed or insufficient dose, survival is poor (Kundrick et al., 2020; Marrero-Rosado, de Araujo Furtado, et al., 2018; Marrero-Rosado et al., 2020). Thus, in addition to effects on seizure severity, we evaluated whether pretreatment with CBD would reduce mortality in soman-exposed Es1−/− mice treated with standard medical countermeasures of atropine, an oxime, and a benzodiazepine.

2. Materials and methods

2.1. Animals

Female Es1−/− mice (8–9 weeks of age; 17–20 g) were obtained from the United States Army Medical Research Institute of Chemical Defense (USAMRICD) breeding colony. Mice were singly housed on a 12-hour:12-hour light-dark cycle with lights on at 0600, with food and water available ad libitum and were weighed daily (M-F). The experimental protocol was approved by the Institute Animal Care and Use Committee at USAMRICD, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the Animal Welfare Act of 1966 (P.L. 89- 544), as amended.

2.2. Surgical implantation of telemetry transmitters for EEG recordings

A subset of mice were implanted with ETA-F10 transmitters from Data Science International (DSI™). Briefly, transmitters were placed subcutaneously (SC) with a wire tunneled and wrapped around a stainless steel screw electrode (1.5 mm lateral, 1.5 anterior and 3.0 posterior to bregma) to record cortical EEG (for detailed methods see (Kundrick et al., 2020)). Mice pre-treated with meloxicam (1 mg/kg, SC) had surgery under 1–5% isoflurane and received post-surgical buprenex SR (0.5 mg/kg, SC). After two weeks of surgical recovery, EEG activity was continuously recorded using Dataquest Art Acquisition (digitized at 250 Hz).

2.3. Soman exposure and medical countermeasures

Soman (pinacolyl methylphosphonofluoridate) was obtained from the U.S. Army Combat Capabilities Development Command Chemical Biological Center and diluted with saline (Hospira). The pharmacokinetic profile of CBD was taken into consideration when determining the time and route of CBD administration in the present study. In mice, intraperitoneal (IP) administration of CBD leads to higher concentrations of the compound in the brain than via the oral route, and the maximum concentration is reached at 60 min following administration (Deiana et al., 2012). Thus, to evaluate the effect of CBD on CWNA-induced seizure severity at a point when the compound is at peak concentration, CBD was administered as a pretreatment in the same manner as studies in other seizure models (Do Val-da Silva et al., 2017; Gobira et al., 2015; Jones et al., 2012; Jones et al., 2010; Klein et al., 2017; Patra et al., 2019). The selection of the dose to be used in our experiments was made based on the reported median effective doses (ED_{50}) in other seizure

models. Klein, et al. (2017) estimated the range of anti-seizure ED_{50}s of CBD in various seizure models to be 83.5–164 mg/kg. Mice were IP administered CBD (20 mg/kg (n=9); 80 mg/kg (n=9); 150 mg/kg (n=13); Cayman) or vehicle (VEH; 5% ethanol [Sigma-Aldrich], 5% Kolliphor EL [Sigma-Aldrich], and 90% saline, IP; n=10) at 60 min prior to soman (80 μg/kg; SC) exposure. An admix (IP) of atropine sulfate (4 mg/kg; Sigma-Aldrich) and HI-6 dichloride (50 mg/kg; Kalexsyn) was administered at 1 min after exposure, and midazolam (5 mg/kg, IP) at 30 min after seizure onset. Control mice (n=6) did not receive soman but instead received saline followed by midazolam 50 min after saline administration.

2.4. Behavioral seizure and EEG analysis

Using Noldus Pocket Observer (Noldus Information Technology), toxic signs were monitored for 4 h after exposure. A modified Racine scale (Racine, 1972) was used to categorize the severity of behavioral seizures in 6 stages: 0, no abnormality; 1, oral movements; 2, head nodding and/or tremors; 3, forelimb clonus or tonus, body tremors; 4, rearing with forelimb clonus; and 5, rearing and falling with convulsions. EEG seizure onset was monitored in real-time and defined as the appearance of rhythmic high amplitude spikes (>2 × baseline) that lasted at least 10 seconds. Seizure events were identified by a MATLAB-based algorithm and visually confirmed or rejected as previously described (Kundrick et al., 2020). Full EEG power spectrum data were further reduced by extracting the median power (20-min bins) in 60-min intervals to obtain EEG power spectral density values at SE (20 min before treatment), and at 1 h, 3 h and 6 h after midazolam treatment. The mean power of delta EEG frequency (0.1–4.0 Hz) was also calculated and integrated in 10-min bins for up to 12 hours according to previous methods (de Araujo Furtado et al., 2009).

2.5. Data analysis

A Kaplan Meier analysis was performed to determine if CBD pre-treatment affected median survival time. Logistic regression analysis was performed to evaluate the effect of treatment on survival at study endpoint, followed by a Chi square and a Fisher's exact test for group comparisons. A generalized linear model analysis with a repeated measure paradigm was used to evaluate treatment effects on percent of change in body weight, power spectral density, and delta power over time, followed by one-way analysis of variance (ANOVA) with Tukey's test at each time point for significant time by treatment interaction. A Kruskal-Wallis test with Bonferroni correction for multiple comparisons was used at each time point to determine the effects of CBD pre-treatment on behavioral seizure severity. Differences were considered statistically significant when $P < 0.05$ using IBM SPSS Statistic v.21 for analysis.

3. Results

3.1. Effects of CBD pre-treatment on survival and body weight following soman exposure

Following CBD pre-treatment and soman exposure plus medical countermeasures, 56%, 33%, and 92% of female mice that received 20 (CBD20-Soman), 80 (CBD80-Soman), or 150 (CBD150-Soman) mg/kg of CBD, respectively, survived the study duration of 3 days, whereas only 29% given a VEH pre-treatment (VEH-Soman) survived (Figure 1A). All

control (SAL) mice that did not receive soman survived. A significant main effect of pre-treatment was detected on median survival time and survival rate at study endpoint. The VEH-Soman group showed a median survival time of 2.5 days (IQR = 2) which was significantly different from SAL group and the CBD150-Soman group, with a median survival time of 3 days (IQR = 0). The median survival time of CBD150-Soman group did not differ from that of the SAL group. A chi square analysis of the ratio of survival at the endpoint revealed that the percentage of surviving animals in the VEH-Soman and CBD80-Soman were significantly reduced compared to that of the SAL group. The 56% survival in the CBD20-Soman group did not differ from that of the SAL group, however it was not significantly higher than the percent of survival of the VEH-Soman group. Only the CBD150-Soman group had a percentage of survival that was not different from that of SAL group and also significantly higher than that of VEH-Soman group. Thus, the survival analyses indicated that the dose of 150 mg/kg of CBD was the only treatment to significantly improve both median survival time and overall percent of survival.

Body weight loss occurred in all mice after soman exposure but to a lesser extent in CBD150-Soman mice (Figure 1B). Soman-exposed mice pre-treated with 150 mg/kg of CBD lost an average (± SD) of 10.8% ± 5.1% body weight, while VEH-Soman mice lost 17.3% ± 4.4%, CBD20-Soman lost 22.5% ± 4.9%, and CBD80-Soman lost 20.4% ± 2.3% within 24 h of exposure. At the 24 h time point after exposure, all soman-exposed animal groups showed significant body weight loss compared to the SAL control group. However, the CBD150 had less weight loss compared to the VEH-treated mice. At 48 h after exposure only mice pre-treated with VEH, CBD20 or CBD80 showed a significant decrease in weight, while the average weight loss in the CBD150-Soman group did not differ from that of the SAL group, thus, demonstrating that the group receiving the highest dose of CBD was able to recover faster in regards to body weight. By 72 hr after exposure, all soman-exposed groups had an average percentage of body weight change that was not different from that of the SAL group.

3.2. Effect of CBD pre-treatment on seizure severity following soman exposure

Because of the greater benefits in survival and body weight, and indicator of overall health, a subset of animals were instrumented with telemetry devices to investigate the effects of the most efficacious CBD dose on seizure severity. Pre-treatment with 150 mg/kg of CBD reduced seizure severity in midazolam-treated mice following soman exposure. In mice with transmitters, soman induced status epilepticus in 3 of 4 VEH-Soman and 4 of 6 CBD150-Soman mice. Seizure onset was within an average (± SD) of 6.0 ± 1.0 min in VEH-Soman mice and 8.0 ± 0.8 min in CBD-Soman mice. Behavioral seizure occurred within an average of 3 min in both soman-exposed groups, reaching a maximal score of 3 in VEH-Soman group and 4 in CBD150-Soman group (Figure 2). Although pre-treatment with CBD did not prevent the development of soman-induced behavioral seizure activity, midazolam treatment given 30 min after seizure onset reduced the seizure severity score in CBD-pretreated mice to values that were not significantly different from the SAL group. In contrast, soman-exposed mice that were pre-treated with VEH continued to show significantly elevated seizure scores compared to SAL group.

EEG power spectral density increased in soman-exposed mice at the time of status epilepticus, albeit with CBD150-Soman mice showing a reduced intensity compared to VEH-Soman mice (Figure 3A). At 1 and 3 h after midazolam treatment, the power spectral density of CBD150-Soman mice was not different from that in the SAL group and was reduced compared to that in VEH-Soman mice suggesting that 150 mg/kg of CBD reduced EEG seizure severity. Soman exposure resulted in an increase in EEG delta power in both soman-exposed groups. However, once midazolam was administered, delta power in CBD150-Soman mice did not differ from SAL mice, while VEH-Soman mice continued to have significantly increased delta (Figure 3B). Thus, although CBD did not prevent seizure in soman-exposed mice, CBD attenuated seizure severity in midazolam-treated mice.

4. Discussion

Our current findings further support the potential of CBD in treating seizures. Pre-treatment with the higher dose of CBD (150 mg/kg) prior to soman exposure greatly increased survival and moderately attenuated seizure severity in midazolam-treated Es1−/− mice. In contrast, midazolam-treated mice that did not receive CBD pre-treatment, as well as those receiving lower doses of CBD, had poor survival to soman exposure, which is in agreement with our previous findings of poor survival in soman-exposed mice with delayed midazolam treatment (Kundrick et al., 2020). The increase in survival in CBD-pre-treated mice is consistent with studies in rat models of mesial temporal lobe epilepsy (Do Val-da Silva et al., 2017) and penicillin-induced partial seizures (Jones et al., 2012), in which CBD pre-treatment increased survival.

The reduction in the EEG power spectral density at the time of status epilepticus in CBD150-Soman mice is thought to indicate reduced seizure severity, as demonstrated in another rodent seizure model (Vučević et al., 2008). Although pre-treatment with CBD prevented seizure onset in several animal models (Lazarini-Lopes et al., 2020), in the lamotrigine-resistant amygdala kindled rat model, CBD was ineffective at reducing behavioral seizure scores and after-discharge duration (Klein et al., 2017). Consistent with a reduction in EEG power density, delta band EEG activity, thought to be a biomarker of neuropathological damage following soman-induced seizure in rats (McDonough et al., 1998; Philippens et al., 1992), was also reduced in CBD150-Soman mice. Similarly to rats, Es1−/− mice also show a robust increase in delta activity following exposure to a seizure-inducing dose of soman (Marrero-Rosado, de Araujo Furtado, et al., 2018; Marrero-Rosado et al., 2020). In the current study, although CBD pre-treatment did not prevent seizure, reduced seizure severity in soman-exposed mice suggests that CBD ameliorates the effects of soman exposure in mice treated with midazolam. While maximum concentration of CBD in brain is reached at 60 min following IP administration (Deiana et al., 2012), midazolam has rapid distribution and onset of action (reviewed in Newmark, 2019) but is less effective when treatment is delayed (Marrero-Rosado et al., 2020); thus, pretreatment with CBD which has relatively slow pharmacokinetic distribution may have potentiated the anti-seizure effects of midazolam treatment 30 min after status epilepticus.

One potential mechanism by which CBD may augment the effects of midazolam is through its effects on $GABA_A$ receptors. Treatment with CBD prevents lethality and tonic seizure

induced by a variety of $GABA_A$ receptor-inhibiting convulsant drugs, but not by a glycine receptor antagonist (Consroe et al., 1982). *In vitro* CBD enhanced the current evoked by GABA, an effect that did not involve the benzodiazepine site (Bakas et al., 2017). Moreover, CBD acts as an allosteric potentiator of extrasynaptic $GABA_A$ receptors. CBD and midazolam, two positive allosteric modulators at different subunits of the $GABA_A$ receptor, may in combination increase the sensitivity to GABA, thereby working synergistically to reduce the severity of seizure.

In addition to the effects on GABA function, CBD may also ameliorate the effects of soman exposure through reduced glutamate release. Increased GABA function and reduced glutamatergic activity are important targets to reduce the effects of cholinergic-induced seizure (Niquet et al., 2020). In a cocaine-induced seizure model, CBD reduces glutamate release in a cannabinoid receptor-independent manner and involving the mammalian target of rapamycin (mTOR) pathway (Gobira et al., 2015). Phosphatydylinositol 3-kinase (PI3K), another member of the mTOR pathway may be necessary for the anticonvulsive effects of CBD as PI3Kγ knockout mice do not show the benefits of increased latency and reduced severity of pilocarpine-induced seizures (Lima et al., 2020). Other mechanisms of CBD include increases in endocannabinoid signaling, as well as effects on calcium mobilization through agonistic effects on vanilloid type 1 (TRPV1) receptors and antagonism of G protein-coupled receptor 55 (GPCR55), which reduces intracellular calcium (Lazarini-Lopes et al., 2020). CBD can also inhibit voltage-dependent sodium currents, thereby modulating neuronal excitability (Billakota et al., 2019; Lazarini-Lopes et al., 2020). Additionally, CBD may control seizures by modulating neuronal and non-neuronal targets via pathways that include anti-neuroinflammatory signaling (Reddy, 2017). Thus, multiple mechanisms of CBD may contribute to its anti-seizure effects.

A pre-treatment protocol was evaluated in the present study since in mice, an IP administration of 120 mg/kg of CBD results in a maximal concentration of the drug in brain tissue after 60 min (Deiana et al., 2012). Reaching a maximum concentration of CBD in the brain may be necessary for the proper evaluation of CBD effects on the severity of soman-induced status epilepticus. However, others have also conducted studies to investigate the disease modifying effects of CBD in a model of temporal lobe epilepsy. In a model of pilocarpine-induced status epilepticus a repeated CBD treatment during the first five days following the toxic insult resulted in a decrease in epileptic behaviors (Hosseinzadeh, Nikseresht et al. 2016). Moreover, anticonvulsant and neuroprotective effects of post-exposure treatment with a potent CBD analogue, HUF-101, following amygdala kindling and pilocarpine-induced seizure suggests potential as a therapeutic (Garcia-Cairasco et al., 2019). Since, similar to the pilocarpine model, we have previously shown that a period of epileptogenesis ensues following soman-induced status epilepticus such that in the days after the insult the animals exhibit spontaneous recurrent seizures (de Araujo Furtado et al., 2010; Kundrick et al., 2020; Marrero-Rosado, de Araujo Furtado, et al., 2018; Marrero-Rosado et al., 2020), more research is warranted to determine the potential of CBD or potent analogues of CBD as a post-exposure treatment to CWNA exposure

In addition to demonstrating the anticonvulsive properties of CBD in various models of seizures, CBD administration (up to 200 mg/kg), a dose higher than that currently used, did

not impair performance in various motor coordination tests in rats (Jones et al., 2012). Moreover, CBD seems to not exert psychotropic or other undesirable effects in humans and is, therefore, well tolerated (Cunha et al., 1980). The strong empirical evidence from various pre-clinical seizure models, including the present soman-exposed Es1−/− mouse model, supporting the benefits of CBD combined with little to no side effects in humans. Clinical studies have demonstrated the antiepileptic benefits of adding CBD to a regimen of conventional antiseizure medications in patients diagnosed with Lennox-Gastaut and Dravet syndromes. In both studies, a therapeutic dose of 20 mg/kg per day of an oral solution of CBD for a period of 14 weeks reduced the frequency of seizures (Devinsky et al., 2017; Devinsky et al., 2018). Estimating a safe starting dose for humans in clinical studies based on the dose of the compound that does not result in adverse effects in pre-clinical animals is commonly done by allometric scaling, a dose-by-factor approach that assumes the metabolic rate of an animal based on body surface area (Nair et al., 2016). Using this method, we estimate that the dose of cannabidiol used in our studies is the human equivalent dose of 12.2 mg/kg, which is similar to the effective doses in clinical studies. However, the route of drug administration (oral in clinical studies, IP in present studies) as well as treatment regimen (once daily in clinical studies, once before exposure in present studies) confound making this comparison. Moreover, it has been argued that it is erroneous to use allometric scaling for the extrapolation of pharmacologically effective doses in pre-clinical animal models to human doses that elicit a therapeutic effect (Blanchard et al., 2015).

In sum, our observations are the first to provide evidence that CBD is beneficial in reducing lethality as well as behavioral and EEG seizure severity stemming from acute nerve agent exposure. Future studies are needed to determine if the current findings extend to male mice or if there are sex differences in response to CBD, as well as to delineate the mechanisms by which CBD improves the outcome to soman exposure. In addition, more potent analogues of CBD should be evaluated for efficacy against soman-induced seizure. Finally, studies on post-exposure efficacy and repeated dosing studies in soman-exposed mice are needed to identify potential therapeutic benefits against CWNA exposure.

Acknowledgement

This research was supported by an Interagency Agreement (AOD16024–001-00000) between NIH/NIAID and the USAMRICD. E.R.K. and B.M.M.R. contributed equally to the manuscript writing and data analysis. The authors acknowledge Ms. Katie Walker for her assistance in data entry, Ms. Robyn Lee-Stubbs for statistical advice, Ms. Cindy Kronman for editorial review and Dr. Linn Cadieux, Ms. Sandra DeBus, and Mr. Timothy Barry, II, for management of the USAMRICD Es1−/− mouse colony. E.R.K. was supported in part by an appointment to the Research Participation Program for the U.S. Army Medical Research and Development Command administered by the Oak Ridge Institute for Science and Education through an agreement between the U.S. Department of Energy and U.S. Army Medical Research and Development Command.

REFERENCES

Asaka Y, Jugloff DG, Zhang L, Eubanks JH, Fitzsimonds RM. Hippocampal synaptic plasticity is impaired in the Mecp2-null mouse model of Rett syndrome. Neurobiol Dis. 2006; 21(1): 217–227. [PubMed: 16087343]

Bakas T, van Nieuwenhuijzen PS, Devenish SO, McGregor IS, Arnold JC, Chebib M. The direct actions of cannabidiol and 2-arachidonoyl glycerol at GABAA receptors. Pharmacol Res. 2017; 119: 358–370. [PubMed: 28249817]

Billakota S, Devinsky O, Marsh E. Cannabinoid therapy in epilepsy. Curr Opin Neurol. 2019; 32(2): 220–226. [PubMed: 30676535]

Blanchard OL, Smoliga JM. Translating dosages from animal models to human clinical trials--revisiting body surface area scaling. FASEB J. 2015; 29(5): 1629–1634. [PubMed: 25657112]

Consroe P, Benedito MA, Leite JR, Carlini EA, Mechoulam R. Effects of cannabidiol on behavioral seizures caused by convulsant drugs or current in mice. Eur J Pharmacol. 1982; 83(3–4): 293–298. [PubMed: 6129147]

Cunha JM, Carlini EA, Pereira AE, Ramos OL, Pimentel C, Gagliardi R, Sanvito WL, Lander N, Mechoulam R. Chronic administration of cannabidiol to healthy volunteers and epileptic patients. Pharmacology. 1980; 21(3): 175–185. [PubMed: 7413719]

de Araujo Furtado M, Lumley LA, Robison C, Tong LC, Lichtenstein S, Yourick DL. Spontaneous recurrent seizures after status epilepticus induced by soman in Sprague-Dawley rats. Epilepsia. 2010; 51(8): 1503–1510. [PubMed: 20067510]

de Araujo Furtado M, Zheng A, Sedigh-Sarvestani M, Lumley L, Lichtenstein S, Yourick D. Analyzing large data sets acquired through telemetry from rats exposed to organophosphorous compounds: an EEG study. J Neurosci Methods. 2009; 184(1): 176–183. [PubMed: 19632275]

Deiana S, Watanabe A, Yamasaki Y, Amada N, Arthur M, Fleming S, Woodcock H, Dorward P, Pigliacampo B, Close S, Platt B, Riedel G. Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Delta(9)-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behaviour. Psychopharmacology (Berl). 2012; 219(3): 859–873. [PubMed: 21796370]

Devinsky O, Cross JH, Laux L, Marsh E, Miller I, Nabbout R, Scheffer IE, Thiele EA, Wright S. Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. N Engl J Med. 2017; 376(21): 2011–2020. [PubMed: 28538134]

Devinsky O, Patel AD, Cross JH, Villanueva V, Wirrell EC, Privitera M, Greenwood SM, Roberts C, Checketts D, VanLandingham KE, Zuberi SM, Group GS. Effect of Cannabidiol on Drop Seizures in the Lennox-Gastaut Syndrome. N Engl J Med. 2018; 378(20): 1888–1897. [PubMed: 29768152]

Do Val-da Silva RA, Peixoto-Santos JE, Kandratavicius L, De Ross JB, Esteves I, De Martinis BS, Alves MN, Scandiuzzi RC, Hallak JE, Zuardi AW, Crippa JA, Leite JP. Protective Effects of Cannabidiol against Seizures and Neuronal Death in a Rat Model of Mesial Temporal Lobe Epilepsy. Front Pharmacol. 2017; 8: 131. [PubMed: 28367124]

Duysen EG, Koentgen F, Williams GR, Timperley CM, Schopfer LM, Cerasoli DM, Lockridge O. Production of ES1 plasma carboxylesterase knockout mice for toxicity studies. Chem Res Toxicol. 2011; 24(11): 1891–1898. [PubMed: 21875074]

Garcia-Cairasco N, Pereira PD, Rodrigues-Santos V, Lima Umeoka EH, Cortes de Oliveira JA, Del Veccio F, da Silva Junior RM. Effects of HUF-101, a Cannabidiol Analogue, in Different Experimental Models of Epilepsy. 2019; Paper presented at the 73rd American Epilepsy Society annual meeting, Abstract #3.105, Baltimore, MD.

Gobira PH, Vilela LR, Goncalves BD, Santos RP, de Oliveira AC, Vieira LB, Aguiar DC, Crippa JA, Moreira FA. Cannabidiol, a Cannabis sativa constituent, inhibits cocaine-induced seizures in mice: Possible role of the mTOR pathway and reduction in glutamate release. Neurotoxicology. 2015; 50: 116–121. [PubMed: 26283212]

Hosseinzadeh M, Nikseresht S, Khodagholi F, Naderi N, Maghsoudi N. Cannabidiol post-treatment alleviates rat epileptic-related behaviors and activates hippocampal cell autophagy pathway along with antioxidant defense in chronic phase of pilocarpine-induced seizure. J Mol Neurosci. 2016; 58(4): 432–440. [PubMed: 26738731]

Jones NA, Glyn SE, Akiyama S, Hill TD, Hill AJ, Weston SE, Burnett MD, Yamasaki Y, Stephens GJ, Whalley BJ, Williams CM. Cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures. Seizure. 2012; 21(5): 344–352. [PubMed: 22520455]

Jones NA, Hill AJ, Smith I, Bevan SA, Williams CM, Whalley BJ, Stephens GJ. Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo. J Pharmacol Exp Ther. 2010; 332(2): 569–577. [PubMed: 19906779]

Klein BD, Jacobson CA, Metcalf CS, Smith MD, Wilcox KS, Hampson AJ, Kehne JH. Evaluation of Cannabidiol in Animal Seizure Models by the Epilepsy Therapy Screening Program (ETSP). Neurochem Res. 2017; 42(7): 1939–1948. [PubMed: 28478594]

Kundrick E, Marrero-Rosado B, Stone M, Schultz C, Walker K, Lee-Stubbs RB, de Araujo Furtado M, Lumley LA. Delayed midazolam dose effects against soman in male and female plasma carboxylesterase knockout mice. Ann N Y Acad Sci. 2020.

Lazarini-Lopes W, Do Val-da Silva RA, da Silva-Junior RMP, Leite JP, Garcia-Cairasco N. The anticonvulsant effects of cannabidiol in experimental models of epileptic seizures: From behavior and mechanisms to clinical insights. Neurosci Biobehav Rev. 2020; 111: 166–182. [PubMed: 31954723]

Lima IVA, Bellozi PMQ, Batista EM, Vilela LR, Brandao IL, Ribeiro FM, Moraes MFD, Moreira FA, de Oliveira ACP. Cannabidiol anticonvulsant effect is mediated by the PI3Kgamma pathway. Neuropharmacology. 2020; 176: 108156.

Marrero-Rosado B, de Araujo Furtado M, Schultz CR, Stone M, Kundrick E, Walker K, O'Brien S, Du F, Lumley LA. Soman-induced status epilepticus, epileptogenesis, and neuropathology in carboxylesterase knockout mice treated with midazolam. Epilepsia. 2018; 59(12): 2206–2218. [PubMed: 30368799]

Marrero-Rosado BM, de Araujo Furtado M, Kundrick ER, Walker KA, Stone MF, Schultz CR, Nguyen DA, Lumley LA. Ketamine as adjunct to midazolam treatment following soman-induced status epilepticus reduces seizure severity, epileptogenesis, and brain pathology in plasma carboxylesterase knockout mice. Epilepsy Behav. 2020; 111: 107229.

McDonough JH Jr., Clark TR, Slone TW Jr., Zoeffel D, Brown K, Kim S, Smith CD. Neural lesions in the rat and their relationship to EEG delta activity following seizures induced by the nerve agent soman. Neurotoxicology. 1998; 19(3): 381–391. [PubMed: 9621344]

Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016; 7(2): 27–31. [PubMed: 27057123]

Newmark J Therapy for acute nerve agent poisoning: An update. Neurol Clin Pract. 2019; 9(4): 337–342. [PubMed: 31583189]

Niquet J, Lumley L, Baldwin R, Rossetti F, Suchomelova L, Naylor D, Estrada IBF, Schultz M, Furtado MA, Wasterlain CG. Rational polytherapy in the treatment of cholinergic seizures. Neurobiol Dis. 2020; 133: 104537.

Patra PH, Barker-Haliski M, White HS, Whalley BJ, Glyn S, Sandhu H, Jones N, Bazelot M, Williams CM, McNeish AJ. Cannabidiol reduces seizures and associated behavioral comorbidities in a range of animal seizure and epilepsy models. Epilepsia. 2019; 60(2): 303–314. [PubMed: 30588604]

Philippens IH, Melchers BP, de Groot DM, Wolthuis OL. Behavioral performance, brain histology, and EEG sequela after immediate combined atropine/diazepam treatment of soman-intoxicated rats. Pharmacol Biochem Behav. 1992; 42(4): 711–719. [PubMed: 1513852]

Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol. 1972; 32(3): 281–294. [PubMed: 4110397]

Reddy DS. The Utility of Cannabidiol in the Treatment of Refractory Epilepsy. Clin Pharmacol Ther. 2017; 101(2): 182–184. [PubMed: 27506704]

Shih T, McDonough JH Jr., Koplovitz I Anticonvulsants for soman-induced seizure activity. J Biomed Sci. 1999; 6(2): 86–96. [PubMed: 10087439]

Szaflarski JP, Bebin EM, Cutter G, DeWolfe J, Dure LS, Gaston TE, Kankirawatana P, Liu Y, Singh R, Standaert DG, Thomas AE, Ver Hoef LW, Program UC. Cannabidiol improves frequency and severity of seizures and reduces adverse events in an open-label add-on prospective study. Epilepsy Behav. 2018; 87: 131–136. [PubMed: 30100226]

Vučević D, Hrnčić D, Radosavljević T, Mladenović D, Rašić-Marković A, Lončar-Stevanović H, Djurić D, Macut D, Šušić V, Stanojlović O. Correlation between electrocorticographic and motor phenomena in lindane-induced experimental epilepsy in rats. Can J Physiol Pharmacol. 2008; 86(4): 173–179. [PubMed: 18418426]

Highlights:

- Cannabidiol pre-treatment reduces lethality to soman exposure in female Es1−/− mice
- Cannabidiol reduces soman-induced seizure severity in midazolam-treated female mice
- Cannabidiol reduces soman-induced weight loss in midazolam-treated female mice
- Cannabidiol may augment the effects of midazolam in mitigating soman-induced toxicity

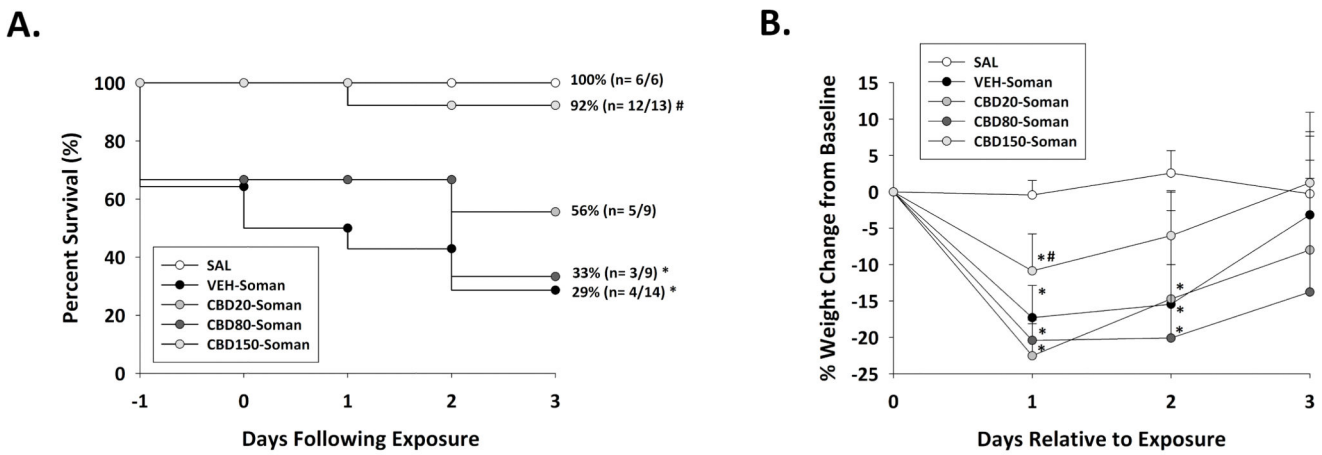

Figure 1. CBD pre-treatment increases survival and attenuates body weight loss following soman-induced seizure and midazolam (3 mg/kg) in female Es1−/− mice.

Mice pre-treated with 20 mg/kg (CBD20-Soman), 80 mg/kg (CBD80-Soman), 150 mg/kg (CBD150-Soman) of CBD or with vehicle (VEH-Soman) at 60 min prior to SC soman (80 μg/kg) exposure were treated with midazolam 30 min after seizure onset. CBD150-Soman group had a median survival time that was not statistically different from saline control (SAL). In contrast, VEH-Soman and CBD80-Soman mice had lower median survival time compared to the SAL control group. The CBD150-Soman group had a significantly larger survival percentage compared to the VEH-Soman group at study endpoint, whereas the CBD20-Soman and CBD80-Soman groups had a survival percentage that was significantly lower than of SAL and did not differ from VEH-Soman; survival percentage of CBD150-Soman group was not significantly different from the SAL group. * $P < 0.05$, compared to SAL; # $P < 0.05$, compared to VEH-Soman. (B) Average percent of change in body weight from pre-exposure baseline is shown. On the first day following exposure, all soman-exposed groups had decreased body weight compared to the SAL group although the CBD150-Soman group had less body weight loss compared to VEH-Soman. By day 2 after exposure, while the VEH-Soman, CBD20-Soman, and CBD80-Soman groups still had decreased body weight compared to the SAL group, the body weight change in the CBD150-Soman group was not significantly different from the SAL group. * $P < 0.05$, compared to SAL group; # $P < 0.05$ compared to VEH-Soman group.

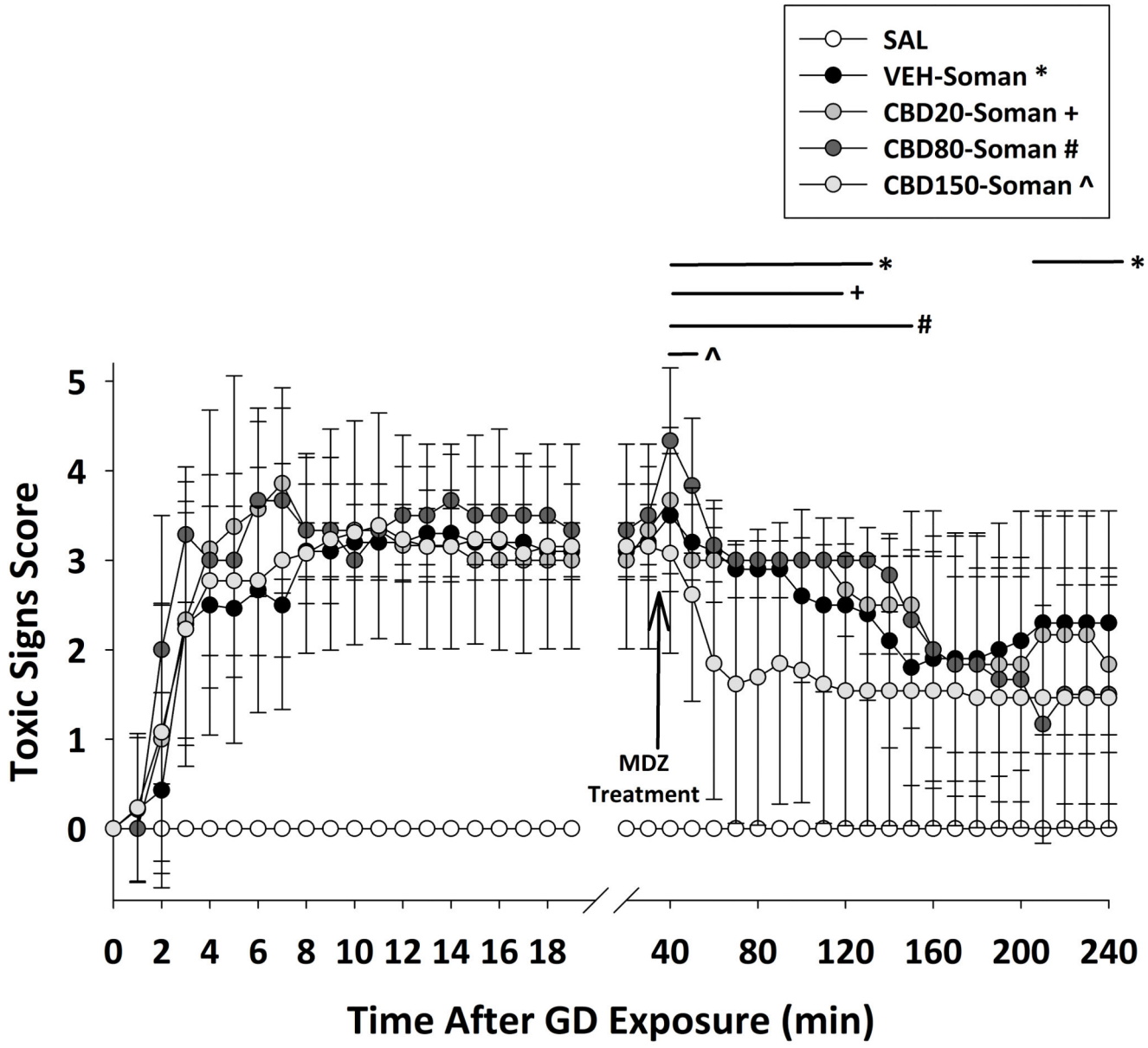

Figure 2. Pretreatment with CBD attenuated behavioral seizure scores in soman-exposed female Es1−/− mice following midazolam (MDZ; 3 mg/kg) treatment.

Mice were pre-treated with 20 mg/kg CBD (CBD20-Soman; n=9), 80 mg/kg CBD (CBD80-Soman; n=9), 150 mg/kg CBD (CBD150-Soman; n=13), or vehicle (VEH-Soman; n=8) 60 min prior to SC exposure to 80 μg/kg of soman followed by MDZ treatment (arrow) at 30 min after seizure onset. Starting at 3 min after exposure, all vehicle- and CBD-treated groups developed toxic signs that were significantly different from saline (SAL) control group. Although all soman-exposed groups developed seizure, seizure in those that received CBD150 pre-treatment was attenuated by MDZ treatment within 20 min of treatment (time 60 min), at which time it was not significantly different from SAL control (^ $P < 0.05$). In contrast, those pre-treated with VEH or lower doses of CBD and treated with midazolam continued behavioral seizure for hours after soman exposure. * $P < 0.05$, VEH-Soman

compared to SAL group; + $P < 0.05$, CBD20-Soman compared to SAL group; # $P < 0.05$, CBD80-Soman compared to SAL group

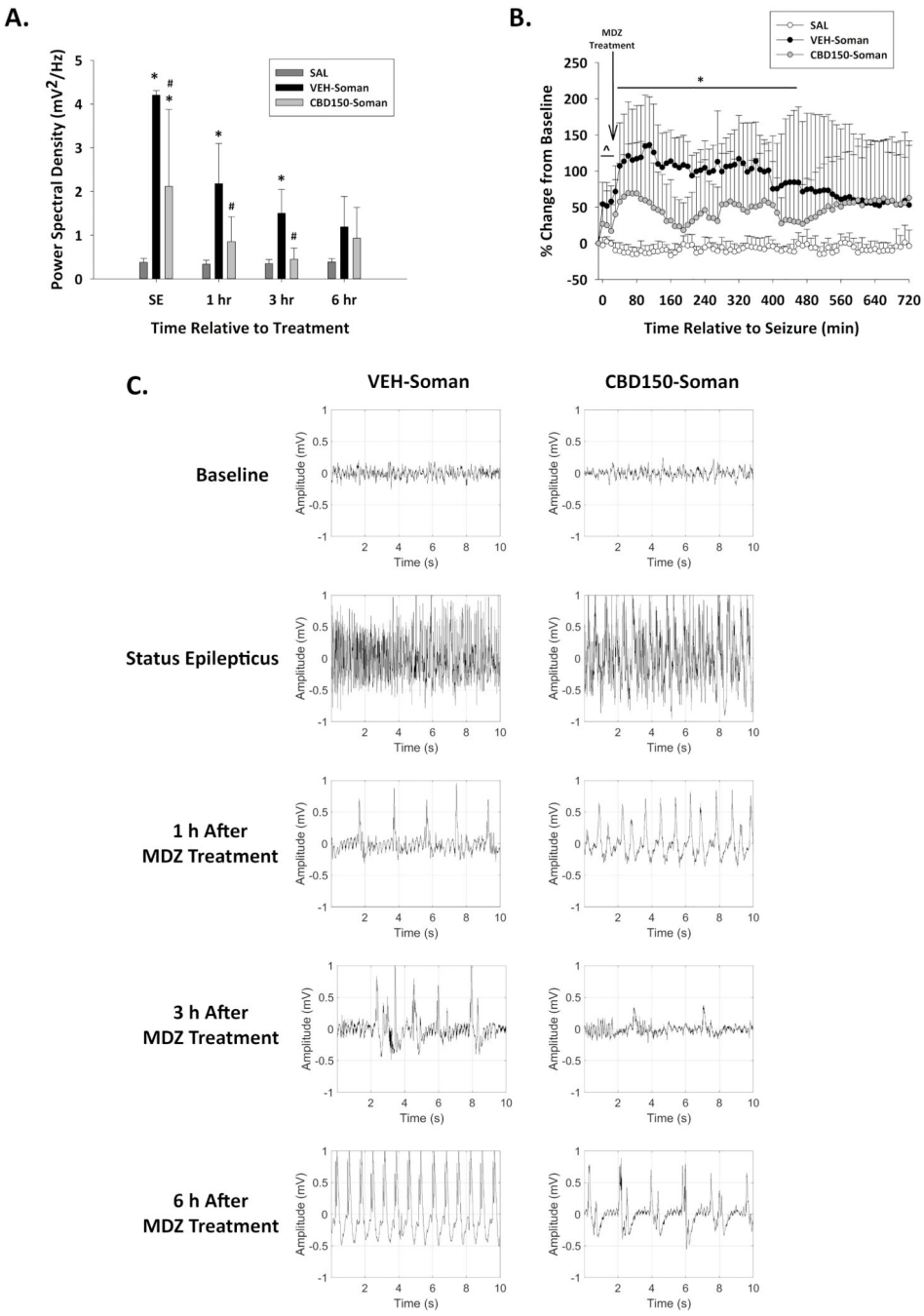

Figure 3. Pre-treatment with CBD attenuated seizure severity following soman exposure and midazolam (MDZ) treatment shown via effects on EEG power spectral density and delta power.

Female Es1−/− mice were pre-treated with CBD (150 mg/kg; CBD150-Soman; n=6) or vehicle (VEH-Soman; n=4) followed by SC exposure to 80 μg/kg of soman and MDZ treatment (arrow) at 30 min after seizure onset. (A) Soman exposure increased EEG power spectral density during SE compared to the SAL group. At 1 and 3 h after treatment, the power density of the CBD150-Soman group had reduced EEG power spectral density compared to the VEH-Soman. (B) Tracing of average percentage of relative change in power of delta (0.1–4 Hz) EEG frequency is shown over a period of 720 min (12 h). In CBD150-

Soman rats, the power of delta did not differ from SAL group following MDZ treatment, while VEH-Soman rats had significantly increased delta power for approximately 440 min following seizure onset. (C) Representative EEG images are shown for baseline (24 h before soman exposure), status epilepticus, and 1, 3, and 6 h after MDZ treatment. *$P < 0.05$, VEH-Soman compared to SAL group; ^ $P < 0.05$, GD compared to SAL; # $P < 0.05$, CBD150-Soman compared to VEH-Soman group.