Randomized blinded controlled clinical trial to assess the effect of oral cannabidiol administration in addition to conventional antiepileptic treatment on seizure frequency in dogs with intractable idiopathic epilepsy

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OBJECTIVE

To assess the effect of oral cannabidiol (CBD) administration in addition to conventional antiepileptic treatment on seizure frequency in dogs with idiopathic epilepsy.

DESIGN

Randomized blinded controlled clinical trial.

ANIMALS

26 client-owned dogs with intractable idiopathic epilepsy.

PROCEDURES

Dogs were randomly assigned to a CBD (n = 12) or placebo (14) group. The CBD group received CBD-infused oil (2.5 mg/kg [1.1 mg/lb], PO) twice daily for 12 weeks in addition to existing antiepileptic treatments, and the placebo group received noninfused oil under the same conditions. Seizure activity, adverse effects, and plasma CBD concentrations were compared between groups.

RESULTS

2 dogs in the CBD group developed ataxia and were withdrawn from the study. After other exclusions, 9 dogs in the CBD group and 7 in the placebo group were included in the analysis. Dogs in the CBD group had a significant (median change, 33%) reduction in seizure frequency, compared with the placebo group. However, the proportion of dogs considered responders to treatment (≥ 50% decrease in seizure activity) was similar between groups. Plasma CBD concentrations were correlated with reduction in seizure frequency. Dogs in the CBD group had a significant increase in serum alkaline phosphatase activity. No adverse behavioral effects were reported by owners.

CONCLUSIONS AND CLINICAL RELEVANCE

Although a significant reduction in seizure frequency was achieved for dogs in the CBD group, the proportion of responders was similar between groups. Given the correlation between plasma CBD concentration and seizure frequency, additional research is warranted to determine whether a higher dosage of CBD would be effective in reducing seizure activity by ≥ 50%. (*J Am Vet Med Assoc* 2019;254:1301–1308)

Idiopathic epilepsy reportedly affects 0.5% to 5.7% of the pet dog population, making it the most common neurologic condition in dogs.[1] A limited number of AEDs are licensed for the treatment of epilepsy in dogs. The most recent American College of Veterinary Internal Medicine consensus statement on seizure management in dogs[2] indicates that anticonvulsant treatment should be initiated with phenobarbital or potassium bromide. However, a combination of phenobarbital and potassium bromide is unsuccessful in controlling seizures in approximately 20% to 30% of dogs.[3] The ineffectiveness and adverse effects of these drugs have caused many dog owners to search for alternative treatments, including cannabis. Although, to the authors' knowledge, no reports have been published regarding the efficacy of cannabis products in the treatment of dogs with idiopathic epilepsy, cannabis products have been anecdotally reported to reduce seizure activity in humans and pets.[4–7]

ABBREVIATIONS

AED	Antiepileptic drug
ALP	Alkaline phosphatase
C-BARQ	Canine Behavioral Assessment and Research Questionnaire
CBD	Cannabidiol
CYP	Cytochrome P450
THC	Tetrahydrocannabinol

More than 104 cannabinoids have been identified as constituents of the *Cannabis sativa* plant. The 2 most abundant cannabinoids are CBD, which is a nonpsychotropic cannabinoid, and THC, which is a psychotropic cannabinoid. Although THC is toxic to dogs, there is hope that CBD may be a safe alternative for medical use. Anticonvulsant properties of CBD have been established in vitro.[8] Cannabidiol does not bind type 1 cannabinoid receptors, but it appears to

have anticonvulsant effects via other mechanisms, including binding to certain transient receptor potential channels, which leads to decreased release of glutamate (a major excitatory neurotransmitter), activation of 5-hydroxytryptophan 1A receptors, and inhibition of adenosine reuptake.[9–12] Preclinical studies[13–15] involving rats and mice with experimentally induced seizures have demonstrated the anticonvulsant effects of CBD.

Recently, a 99% pure CBD medication formulated for oral administration was approved by the US FDA for treatment-resistant epilepsy in humans.[16] During the approval process for that product, the US Drug Enforcement Administration was provided with a medical and scientific analysis of CBD so that it could reevaluate use of the product and make a scheduling determination. Subsequently, the Drug Enforcement Administration rescheduled FDA-approved CBD products as a schedule V substance.

Because of its nonpsychoactive characteristics, lack of reported adverse effects, and anticonvulsive properties, CBD has potential for use as an AED.[4,8,17,18] The purpose of the study reported here was to assess the short-term effect of addition of CBD to standard AED treatment on seizure frequency in dogs with intractable idiopathic epilepsy. Secondary objectives included evaluation of the effect of CBD on serum phenobarbital and bromide concentrations, measurement of the plasma CBD concentrations over a 12-week oral administration period, and identification of any adverse clinical and clinicopathologic effects.

Materials and Methods

Animals

Dog owners contacted the neurology service of the Colorado State University veterinary teaching hospital after finding our clinical trial advertisement on the university's website or hearing about it through word of mouth (eg, via the primary care veterinarian or other pet owners). The goal was to enroll 30 dogs (15/group) over a maximum of 2 years. Dogs were included in the study if they met the tier II confidence level for diagnosis of idiopathic epilepsy.[19] Specifically, dogs were required to have had ≥ 2 seizures/mo for at least 16 weeks (and owners were required to provide documentation of this) while being treated with a minimum of 1 conventional AED, no abnormalities identified on routine clinicopathologic testing (CBC and serum biochemical analysis) that would account for the seizures, unremarkable findings on MRI of the brain and CSF analysis, and negative results of infectious disease testing (for *Bartonella* spp, *Ehrlichia* spp, *Anaplasma* spp, *Neorickettsia* spp, *Dirofilaria immitis*, *Wolbachia* spp, *Neospora caninum*, *Rickettsia* spp, *Toxoplasma gondii*, *Neospora caninum*, *Borrelia burgdorferi*, and canine distemper virus).

Additionally, all dogs were required to either have had a serum phenobarbital or bromide concentration within a therapeutic range[20–22] of 20 to 40 µg/mL for phenobarbital and 0.67 to 2 mg/mL for bromide or be receiving the labeled dose of zonisamide (≥ 5 mg/kg [2.3 mg/lb], PO, q 12 h) or levetiracetam (immediate-release formulation, > 20 mg/kg [9.1 mg/lb], PO, q 8 h; extended-release formulation, ≥ 30 mg/kg [13.6 mg/lb], PO, q 12 h). The AED protocol initiated at the start of the study remained the same throughout the study period. Dogs were excluded from the study if they had a comorbidity associated with a poor prognosis.

Study design

Performed at the veterinary teaching hospital at Colorado State University from 2016 to 2017, the study was designed as a randomized blinded controlled clinical trial comparing the effect of CBD versus a placebo on seizure frequency when added to standard AED treatment. Dogs were randomly assigned to a CBD group or placebo group by an individual not directly involved in the study who used a computer-based random number generator for this assignment. All veterinary personnel directly involved with the study and all dog owners were unaware of the group assignment.

Before assigned medications were dispensed for administration by owners at home, dogs underwent a diagnostic workup to rule out causes of seizures other than idiopathic epilepsy. Owners of dogs in the CBD group were then provided with CBD-infused oil[a] to be orally administered twice daily at 2.5 mg/kg (1.1 mg/lb) for 12 weeks in addition to currently prescribed AEDs, and owners of dogs in the placebo group were provided the same oil without CBD to be administered under the same conditions.

Test products

Each batch of CBD-infused oil was analyzed at a third-party laboratory[b] and verified at Colorado State University's core pharmacology laboratory. The concentrations of CBD, THC, cannabinol, cannabigerol, and cannabichromene were measured by use of a triple quadrupole mass spectrometer.[c] The whole-plant CBD-infused oil contained 100 mg of CBD/mL along with trace amounts of the other cannabinoids. The remaining ingredients were cold-pressed hemp oil and oil-miscible chicken flavoring. The placebo contained only cold-pressed hemp oil (without cannabinoids) and oil-miscible chicken flavoring. The 2 products were indistinguishable in appearance and scent.

The sourced low-THC cannabis plants were registered with and certified by the Colorado Department of Agriculture as containing < 0.3% THC on a dry weight basis, which, according to the Agricultural Act of 2014 (also known as the Farm Bill), is by definition considered industrial hemp. The Farm Bill allows individuals and institutions to participate in research with industrial hemp in states where such activity is legal.[23] The legal status under federal law of CBD derived from industrial hemp is currently unclear. Cannabidiol derived from high-THC cannabis is considered a schedule I substance by the Controlled Sub-

stances Act and is therefore not considered legal for use by the US Drug Enforcement Administration. Cannabidiol derived from portions of the cannabis plant not considered marijuana is exempt from schedule I.

Evaluation of effects

Owners were asked to return their dogs every 4 weeks for blood sample collection and monitoring of plasma CBD concentration. Blood samples were also collected before (week 0) and at the end of the 12-week study period for a CBC, serum biochemical analysis, and serum phenobarbital or bromide concentration measurement. Neurologic and physical examinations were repeated at the final visit (week 12).

Throughout the study, owners were asked to keep a standardized daily seizure log and record the number of seizures, seizure type, and seizure duration for their dogs. When no cluster seizures (ie, > 1 seizure within a 24-hour period) were noted, each seizure was counted individually. In the event of cluster seizures, each cluster was counted as 1 seizure episode. For example, if a dog had 3 seizures within 24 hours, this was counted as 1 seizure episode. Mean monthly seizure frequency was calculated for each dog by consideration of the monthly number of seizures during the 16-week period before the study began and the monthly number of seizures during the 12-week study period. Dogs were considered to have had a response to treatment if they had a ≥ 50% reduction in mean monthly seizure frequency from before the study began to the time the study concluded.

Owners were also asked to complete a validated behavioral questionnaire, the C-BARQ,[24–26] at weeks 0 and 12 to monitor for adverse drug effects and document any changes in anxiety-related behavior with the addition of the CBD-infused oil. This instrument has also been validated to measure neurobehavioral traits in dogs.[27–29] Owners were asked 100 questions related to the behavior of their dogs before and at the end of treatment.

Blood sample collection and testing

Blood samples were collected from a cephalic, saphenous, or jugular vein via 20-gauge needles into plain evacuated tubes (serum biochemical analysis and serum AED concentration), EDTA tubes (CBC), and lithium heparin tubes (plasma CBD concentration). Blood samples for CBC and serum biochemical analysis were submitted to the clinical pathology laboratory at Colorado State University for analysis. Samples for measurement of serum AED concentration were submitted to the diagnostic medical center at the university for analysis.

Samples for measurement of plasma CBD concentration were submitted to the Pharmacology Shared Resource laboratory for analysis. For this analysis, blood samples were first centrifuged at 2,000 × *g* for 10 minutes at 4°C, and harvested plasma was frozen at –80°C until analysis. Cannabidiol concentration was measured in thawed plasma samples by use of a validated liquid chromatography–tandem mass spectrometry assay. Briefly, 50 µL of plasma, standard, or quality control sample was added to a 1.5-mL microcentrifuge tube, and then 5 µL of a deuterated internal CBD standard (100 ng/mL) was added, followed by 50 µL of acetonitrile. Samples were vortex mixed for 5 minutes and centrifuged at 13,300 × *g* for 10 minutes. The resulting supernatant was transferred to an autosampler vial for analysis with a triple quadrupole mass spectrometer[c] and high-performance liquid chromatography system.[d,e] Introduced samples (30 µL) were separated on a C18 column[f] with filter cartridge[g] by use of a mobile phase consisting of 70% solvent A (acetonitrile with 0.1% formic acid) and 30% solvent B (0.1% formic acid in ultrapure water[h]) for 1.5 minutes, followed by a linear gradient to 99% solvent A over 2 minutes; samples were then held for another 2 minutes prior to returning to the starting mobile phase. Multiple reaction monitoring was carried out in positive ion mode with first and second stages (Q1 and Q3) operating at unit resolution with ion spray voltage set to 5,500 V, source temperature set to 550°C, and other instrument parameters optimized to measure transitions of CBD from an m/z of 315.1 to 193.5 and from 315.1 to 259.3 as well as transitions of deuterated CBD from an m/z of 318.4 to 196.8 and from 318.4 to 262.6. Quantification was performed by use of summed values of the measured transitions for CBD corrected for deuterated CBD.

Performance of the assay was linear from the lower limit of quantification of 1 ng/mL to 10 µg/mL, and results for quality control samples indicated that accuracy and precision (coefficient of variation) of the assay were 93.1% and 4.6%, respectively.

Statistical analysis

Two outcome variables related to effectiveness of the evaluated CBD-infused oil were assessed. The median change in mean monthly seizure frequency from before the study began to the time it concluded was compared between the CBD and placebo groups by use of the Wilcoxon signed rank test. Proportions of dogs classified as responders were compared between groups by use of the Fisher exact test.

The Wilcoxon signed rank test was also used for between- and within-group comparisons involving serum biochemical and CBC data before study treatment began (week 0) and after it concluded (week 12). Between-group comparisons of the distributions of various seizure types, laboratory changes (with changes scored as clinically important or normal), and reproductive status were performed with the Fisher exact test. The Wilcoxon 2-sample test was used to compare nonparametric continuous data such as age and body weight between treatment groups. The Kruskal-Wallis test was used for within- and between-group comparisons of C-BARQ scores between the 2 assessment points. Pearson correlation coefficients (*r*) were computed to determine the correlation between plasma CBD concentration and mean change in seizure fre-

quency. The statistician (SR) was blinded to the nature of the group that dogs had been assigned to (coded as group A or B) throughout the data analysis process. Values of $P < 0.05$ were considered significant. Statistical software was used for all analyses.[i]

Results

Animals

Two hundred forty-eight dogs were assessed for eligibility for the study, of which 222 (90%) were excluded because they did not meet the inclusion criteria or the owner declined participation (**Supplementary Figure S1**, available at avmajournals.avma.org/doi/suppl/10.2460/javma.254.11.1301).

Twenty-six dogs were consequently enrolled in the study and randomly assigned to the CBD group (n = 12) or the placebo group (14). Seventeen of the 26 (65%) enrolled dogs completed the study (9 in the CBD group and 8 in the placebo group). Reasons for withdrawal for the other 9 dogs included AED adjustments (n = 3 dogs in the placebo group), owner's inability to return for scheduled appointments (3 dogs in the placebo group), general proprioceptive ataxia (2 dogs in the CBD group), and euthanasia due to status epilepticus 8 weeks after study treatment began (1 dog in the CBD group). For 1 dog with ataxia, the ataxia resolved after CBD administration was discontinued. The other ataxic dog was euthanized owing to seizure activity, which occurred several days after CBD administration was discontinued.

Data from 16 of the 17 dogs (9 in the CBD group and 7 in the placebo group) that completed the study were included in the analysis portion of the study. The owner of the remaining dog (in the placebo group) reported giving the dog CBD-infused oil during the final month of the study; therefore, that dog was excluded from analysis.

The 16 dogs included 11 castrated males, 3 spayed females, and 2 sexually intact males. Median age at the start of the study treatment was 5 years (range, 2 to 12 years), and mean body weight was 29 kg (64 lb; range, 6 to 64 kg [13 to 141 lb]). There were 5 mixed-breed dogs, 2 Golden Retrievers, 1 Pug, 1 Newfoundland, 1 Wirehaired Pointing Griffon, 1 Vizsla, 1 Shetland Sheepdog, 1 Australian Shepherd, 1 Boxer, 1 Dogo Argentino, and 1 Labrador Retriever. Eleven dogs had generalized seizures (6/9 dogs in the CBD group and 5/7 dogs in the placebo group), and 5 dogs had both generalized and focal seizures (3/9 dogs in the CBD group and 2/7 dogs in the placebo group). No significant differences regarding age, reproductive status, body weight, and seizure type were identified between the placebo and CBD groups **(Table 1)**.

Concurrent AED treatment included phenobarbital alone (n = 3); phenobarbital, potassium bromide, and levetiracetam (3); phenobarbital and levetiracetam (2); phenobarbital and zonisamide (2); zonisamide alone (1); potassium bromide, levetiracetam, and zonisamide (1); potassium bromide and zonisamide (1); phenobarbital and potassium bromide (1); phenobarbital, levetiracetam, and zonisamide (1); and potassium bromide and levetiracetam (1).

Treatment effects

Following study treatment, a significant ($P = 0.01$) reduction was identified in the group median for

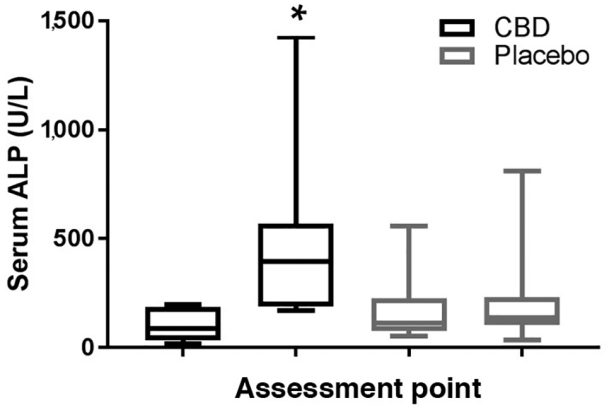

Figure 1—Box-and-whisker plots of serum ALP activity at week 0 (before study treatment) and week 12 for client-owned dogs with intractable idiopathic epilepsy that were randomly assigned to receive CBD-infused oil (2.5 mg/kg [1.1 mg/lb], PO, twice daily for 12 weeks; n = 9; black boxes) or a placebo at a similar dosage (7; gray boxes), in addition to currently prescribed conventional AEDs. The top and bottom of each box represent the 75th and 25th percentiles, respectively; the central horizontal line within each box represents the median; and the whiskers represent the minimum and maximum values. *Values differ significantly ($P = 0.004$) between assessment points for dogs in the CBD group.

Table 1—Characteristics of client-owned dogs with intractable idiopathic epilepsy that were randomly assigned to receive CBD-infused oil (2.5 mg/kg [1.1 mg/lb], PO, twice daily for 12 weeks; n = 9) or a placebo at a similar dosage (7), in addition to currently prescribed conventional AEDs.

Characteristic	CBD group	Placebo group	*P* value
Median (range) age at start of study (y)	5 (2–12)	6 (3–10)	0.16
No. of dogs by reproductive status			> 0.99
Castrated male	5	6	—
Sexually intact male	2	0	—
Spayed female	2	1	—
Median (range) body weight (kg)	25 (6–40)	33 (18–64)	> 0.99
No. of dogs by seizure type			> 0.99
Generalized	6	5	—
Generalized and partial	3	2	—

Table 2—Mean (SD) serum phenobarbital (µg/mL) and bromide (mg/mL) concentrations in the dogs of Table 1 before (week 0) and after (week 12) study treatment and percentage change in values between assessment points for dogs with increases or decreases.

AED and group	Week 0	Week 12	*P* value	Percentage increase from week 0 (No. of dogs with increase)	Percentage decrease from week 0 (No. of dogs with decrease)
Phenobarbital					
CBD (n = 7)	28.4 (4.7)	31.5 (7.8)	0.30	22 (5)	14 (2)
Placebo (n = 4)	33.4 (4.2)	29.5 (6.9)	0.25	16 (1)	26 (3)
Bromide					
CBD (n = 3)	1.2 (0.5)	1.2 (0.5)	1.00	77 (1)	26 (2)
Placebo (n = 2)	1.5 (0.6)	2.0 (0.4)	1.00	112 (1)	10 (1)

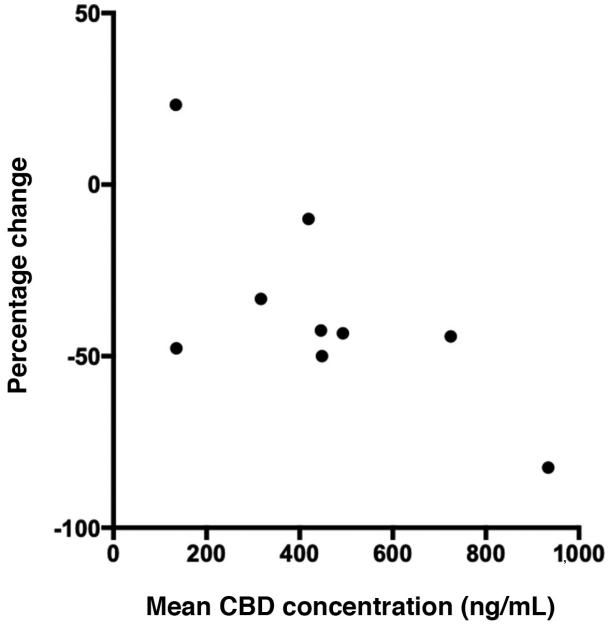

Figure 2—Dot plot showing the negative correlation (r = –0.68; P = 0.04) between mean plasma CBD concentration (measured at weeks 4, 8, and 12) and the percentage change from before study treatment began (week 0) in mean monthly seizure frequency for dogs in the CBD group (n = 9).

mean monthly seizure frequency in the CBD group (33%; median value before and after study treatment, 4.0 and 2.7, respectively), compared with that in the placebo group (0%; 2.0 and 2.0, respectively). Two dogs in the CBD group were classified as responders, compared with 2 dogs in the placebo group (P > 0.99; **Supplementary Table S1**, available at avmajournals.avma.org/doi/suppl/10.2460/javma.254.11.1301).

The only clinicopathologic abnormality noted during the study period was a significant (P = 0.004) increase in serum ALP activity in the CBD group from weeks 0 to 12, compared with activity in the placebo group over the same period **(Figure 1)**. For the dogs with available data, no significant difference in serum phenobarbital or bromide concentration between assessment points was identified within either group, nor was a significant difference identified between the groups at week 0 or 12 **(Table 2)**.

Plasma CBD concentrations were measured at weeks 4, 8, and 12 for all 16 dogs included in the analysis. For the dogs in the CBD group (**Supplementary Table S2**, available at avmajournals.avma.org/doi/suppl/10.2460/javma.254.11.1301), mean plasma CBD concentration over the 3 assessment points was negatively correlated with the percentage change from week 0 in mean seizure frequency (r = –0.68; P = 0.04; **Figure 2**). Except for 1 dog at 1 assessment point, all dogs in the placebo group had no detectable plasma CBD concentration. The plasma CBD concentration for that 1 dog in the placebo group was 6.4 ng/mL at week 8. When the C-BARQ scores for aggression, rivalry, fear, anxiety, trainability, excitability, and energy level at weeks 0 and 12 were compared within and between groups, no significant differences were identified in any of these categories.

Discussion

In the present study, a significant reduction in seizure frequency was achieved in dogs with intractable idiopathic epilepsy receiving CBD-infused oil as administered, compared with findings for dogs in the placebo group. Although the sample size was small, it was interesting to note the significant, negative correlation between percentage change in seizure frequency and plasma CBD concentration. However, no significant difference was identified between treatment groups when the proportion of responders (≥ 50% decrease in seizure activity), which was perhaps a more clinically relevant outcome variable, was compared between groups.

To the authors' knowledge, the present study was the first to evaluate the anticonvulsant effects of CBD in dogs with epilepsy. A study[14] involving mice showed that CBD has anticonvulsant effects against maximal electroshock seizures and tonic seizures induced by convulsant agents such as picrotoxin, 3-mercaptoproprionic acid, pentylenetetrazol, isonicotinic acid hydrazine, and bicuculline. Results of another study[13] suggest that oral administration of CBD to rats was effective in blocking maximal electroshock (at a median effective dose of 12 mg/kg [5.5 mg/lb]) and audiogenic seizures (at a median effective dose of 17 mg/kg [7.7 mg/lb]). In the same study,[13] the anticon-

vulsant potency of CBD in the maximal electroshock test and for audiogenic seizures was only slightly less than that of phenobarbital, which is the most effective AED in dogs.[2]

Since the completion of our clinical trial in dogs, several reports[18,30–34] have been published of clinical trials of efficacy, safety, and tolerability of oral CBD treatment for drug-resistant seizures in humans, including patients with Dravet syndrome and Lennox-Gastaut syndrome (2 rare forms of epilepsy). Those trials showed that CBD is well tolerated and efficacious in humans at dosages ranging from 2 to 20 mg/kg/d (0.9 to 9.0 mg/lb/d).

Testing the effect of a novel product on seizure frequency poses several challenges. Without previously established data in human or veterinary medicine for effective doses, proper dosing intervals, or therapeutic blood concentrations, identification of an appropriate dosing regimen can be difficult. Although the number of responders to the dosage of CBD (2.5 mg/kg, PO, twice daily) in the present study was low (2/9 dogs), the significant reduction in mean monthly seizure frequency in that group was promising. That dosage was based on the limited information available from studies of the effectiveness of CBD as an anticonvulsant. One such study[35] was a human-based trial in which CBD was administered at 1.5 mg/kg (0.7 mg/lb), PO, every 12 hours. Four of 8 participants in that study had "considerable improvement." For the present study, we attempted to convert the 1.5-mg/kg dose for humans to a dog-equivalent dose by use of the following formula[36]:

$$\text{Dog-equivalent dose} = \text{human dose}/(\text{dog } K_m/\text{human } K_m)$$

where K_m represents a correction factor estimated by dividing the mean body weight of a given species by its body surface area. This calculation yielded a dog-equivalent dose of 2.8 mg/kg (1.3 mg/lb), and a CBD dose of 2.5 mg/kg was therefore chosen for evaluation. To further support that choice, findings of a recent CBD pharmacokinetic study[18] in dogs indicated that, even at the predicted values for a dose of 2.5 mg/kg, mean plasma CBD concentrations (1µM) were within the range of in vitro values that attenuate epileptiform activity (0.01µM to 100µM).[8] The twice-daily administration regimen was chosen for the present study on the basis of previously reported findings for humans,[30,31,34] ongoing clinical trials, and canine pharmacokinetic data.[18] The half-life of CBD in dogs that receive a single dose (5 or 10 mg/kg [2.3 to 4.5 mg/lb]) of orally administered CBD-infused oil is reportedly 127.5 to 199.7 minutes,[18] supporting administration at a frequency of no less than twice daily.

In 2018, a highly purified oral CBD formulation was approved by the FDA for use in the treatment of Dravet syndrome and Lennox-Gastaut syndrome in humans.[16] The recommended starting dosage is 2.5 mg/kg, every 12 hours, and can be increased to a maximum dosage of 10 mg/kg, every 12 hours. The dog equivalent of human doses of 2.5 to 10 mg/kg is 4.5 to 18 mg/kg (2.0 to 8.2 mg/lb).[36] Therefore, it is possible that the dosage used for dogs in the present study (2.5 mg/kg, PO, twice daily) was too low given the intractable nature of their epilepsy, resulting in few dogs qualifying as responders. This possibility was further supported by the negative correlation observed between mean plasma CBD concentration and percentage change in mean seizure frequency. For future clinical trials, a dose-escalation design should be considered.

In the study reported here, 1 dog in the placebo group had CBD detected in its plasma sample (6.4 ng/mL) at week 8. The reason for this remains unclear. The owner reported feeling certain that the dog was not exposed to other sources of CBD. It is possible that the placebo oil contained trace amounts of CBD; that product was not tested. However, 3 additional dogs received that same batch of oil throughout the study and had no detectable CBD in their plasma samples at any assessment point. The plasma CBD concentration in this dog at week 8 (6.4 ng/mL) was much lower than the overall mean plasma CBD concentration in the dogs in the CBD group (450.1 ng/mL). Therefore, the trace CBD detected in this dog's plasma sample likely had no influence on seizure frequency.

Adverse effects led to the withdrawal of 3 of the 12 dogs originally assigned to the CBD group and none in the placebo group. The owner of 1 dog in the CBD group chose to have the dog euthanized because it developed status epilepticus. Status epilepticus and cluster seizures are common sequelae to epilepsy[1]; therefore, the status epilepticus in that dog was likely attributable to its seizure condition rather than the CBD. Indeed, to the authors' knowledge, CBD has no reported proconvulsive effects. The other 2 dogs were withdrawn from the CBD group because they developed ataxia. Although none of the other dogs that completed the study had ataxia reported, ataxia should be noted as a potential adverse effect of CBD administration to dogs. Commonly reported adverse effects of CBD treatment in humans participating in clinical trials include somnolence, loss of appetite, and diarrhea.[30–34] None of these effects were reported in the present study.

In humans and possibly other animals, CBD is metabolized by the CYP system in the liver and inhibits several isoenzymes, including CYP 2C19, CYP 2D6, and CYP 2C9, so speculation exists that CBD could affect the metabolism of certain AEDs. In a study[37] of the effect of oral CBD administration on serum concentrations of common AEDs in humans, a starting dosage of 2.5 mg/kg, every 12 hours, up to a maximum dosage of 50 mg/kg/d (22.7 mg/lb/d) had no effect on serum concentrations of phenobarbital or levetiracetam but did significantly affect serum concentrations of clobazam, topiramate, zonisamide (in adults only), eslicarbazepine, and rufinamide. Similarly, in the present study, no significant change in serum phenobarbital concentration was identified in dogs following CBD treatment. Of the AEDs

affected by CBD coadministration in humans, the only one routinely used in veterinary medicine to our knowledge is zonisamide. In the first part of the aforementioned human study,[37] a subset of adults (n = 7) had a significant increase in serum zonisamide concentration with increasing doses of CBD. Further analysis was performed to account for AED adjustments during the study, revealing that the degree of change in zonisamide concentration over time was not significant. In humans, CBD can have inhibitory effects on enzymes involved in the metabolism of zonisamide, specifically CYP 3A4 and *N*-acetyltransferase,[38] which may explain the increase in serum zonisamide concentration observed in that study.[37] Because no therapeutic range has been established for zonisamide in dogs, serum concentrations of that AED have not been routinely tested at our institution. Therefore, zonisamide concentrations were not measured in our study but should be considered for future veterinary studies.

Serum ALP activity increased significantly from weeks 0 to 12 in dogs in the CBD group, compared with activity in dogs in the placebo group. This increase, observed in all 9 dogs in the CBD group, was likely due to the induction of CYP isoenzymes in the liver.[39,40] In humans, phytocannabinoids are extensively metabolized by hepatic CYP enzymes, and CBD is a potent inhibitor of CYP enzymes.[41] Although it is unknown whether this occurs in dogs, it is reasonable to suspect the same holds true. This inhibition may be linked to adverse effects from coadministration of common AEDs, such as potentiation of benzodiazepines and phenobarbital.[42,43] Thus, although the clinical importance of the increase in serum ALP activity observed in the present study remains unknown, other considerations involving drug interactions are of importance. No measurement of serum bile acids concentrations was performed, which would have helped elucidate whether any functional changes to the liver had occurred after 12 weeks of CBD treatment.

A limitation of the study reported here was the small sample size, which made the clinical importance of the observed data difficult to interpret. Given the results, a larger randomized blinded controlled clinical trial involving a higher dose of CBD is warranted. Another limitation inherent to clinical trials involving dogs with epilepsy is the reliance on dog owners for recording outcomes such as seizure frequency. Some seizures could have been missed if they were nocturnal or the owner was absent; however, because owners were unaware of which treatment their dogs received, there was no reason to believe that any bias introduced by misclassification of seizure frequency would be different between treatment groups.

Overall, results of the clinical trial reported here indicated that a significant reduction in seizure frequency was achieved in dogs with intractable idiopathic epilepsy by addition of oral CBD treatment to conventional AED treatment. However, the proportion of dogs with a response to treatment as defined was statistically similar between treatment groups. Additional research is necessary to determine the effect of oral CBD administration on seizure frequency in dogs with epilepsy.

Acknowledgments

Funded by Applied Basic Science Corporation, the company that produced the CBD-infused oil evaluated in the study. This sponsor had no role in study design, data collection and analysis, or preparation of the manuscript.

Dr. McGrath has a 5% ownership in Applied Basic Science Corporation. The authors declare that there were no other conflicts of interest.

Footnotes

a. Colorado Hemp Oil, Applied Basic Science Corp, Denver, Colo.
b. Botanacor Services, Denver, Colo.
c. 3200 QTrap, AB Sciex, Redwood City, Calif.
d. Agilent 1200 series liquid chromatography system, Agilent Technologies Inc, Santa Clara, Calif.
e. HTC-PAL Leap autosampler, Leap Technologies, Carrboro, NC.
f. Waters Sunfire C18 column (4.6 X 50 mm), Waters Corp, Milford, Mass.
g. Phenomenex C18 filter frit guard cartridge, Phenomenex, Torrance, Calif.
h. Milli-Q water, MilliporeSigma, Burlington, Mass.
i. SAS, version 9.4, SAS Institute Inc, Cary, NC.

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