Randomized, placebo-controlled, 28-day safety and pharmacokinetics evaluation of repeated oral cannabidiol administration in healthy dogs

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OBJECTIVE
To determine the safety and pharmacokinetics of various doses of plant-derived cannabidiol (CBD) versus placebo following repeated oral administration.

ANIMALS
20 healthy adult Beagles.

PROCEDURES
In a randomized, blinded, placebo-controlled trial, dogs were randomized to 5 groups balanced in body weight and sex (n = 4 dogs/group) and received a CBD (1, 2, 4, or 12 mg/kg; from cannabis extract) or placebo oil formulation PO once daily for 28 days. Outcome variables were assessed through daily health observations, veterinary examinations, CBC, and serum biochemical analysis. Blood samples were collected at various time points to estimate 24-hour pharmacokinetic profiles of CBD and selected metabolites (7-carboxy-CBD and 7-hydroxy-CBD).

RESULTS
Repeated CBD administration was well tolerated by dogs, with no clinically important changes in measured safety outcomes. Veterinary examinations revealed no clinically important abnormal findings. Adverse events were mild in severity. Relative to placebo administration, CBD administration at 12 mg/kg/d resulted in more gastrointestinal adverse events (mainly hypersalivation) and significantly higher serum alkaline phosphatase activity. Total systemic exposure to CBD increased on a dose-dependent basis following both acute (first dose) and chronic (28 days) administration. Within each CBD dose group, repeated administration increased total systemic exposure to CBD 1.6- to 3.3-fold. The 24-hour trough plasma CBD concentrations were also dose dependent, with a steady state reached following 2 weeks of administration.

CONCLUSIONS AND CLINICAL RELEVANCE
Repeated, daily oral administration of the CBD formulation led to dose-dependent increases in total systemic exposure to CBD and 24-hour trough plasma concentrations in healthy dogs. These findings could help guide dose selection. (*Am J Vet Res* 2021;82:405–416)

Interest is increasing in the potential therapeutic uses of cannabinoids, particularly CBD, in veterinary species.[1-3] Safety and tolerability studies reveal that, in dogs, CBD generally has a favorable safety profile and is well tolerated when administered for 1 to 3 months at dosages ranging from 0.5 to 1.2 mg/kg once daily[4] or 2 to 10 mg/kg twice daily (4 to 20 mg/kg/d)[5-8] or as 10 escalating doses (2 to 62 mg/kg/dose, with 3 days between doses).[9] Reported adverse effects include increases in serum ALP activity[6-9] and gastrointestinal signs, most often observed as loose feces.[5,7]

To the authors' knowledge, only 3 publications have included data on systemic CBD concentrations in dogs following repeated oral administration for 6 weeks at 5 mg/kg/d (CBD in hemp seed oil)[10] or 12 weeks at 10 or 20 mg/kg/d (plant-derived CBD in hemp oil)[6] or dose escalation to approximately 62 mg/kg (plant-derived CBD in MCT oil).[9] Importantly, no studies have been reported regarding systemic CBD concentrations following repeated administration of lower daily doses (< 5 mg/kg), which may be of interest for therapeutic purposes. Furthermore, although the 12-hour[10] or 24-hour[4,7] pharmacokinetic profile of CBD has been explored in dogs following oral administration of a single dose of CBD in hemp

ABBREVIATIONS

7-COOH-CBD	7-carboxy-cannabidiol
7-OH-CBD	7-hydroxy-cannabidiol
AE	Adverse event
ALP	Alkaline phosphatase
AUC_{0-last}	Area under the concentration-versus-time curve from time 0 to the last measured concentration
CBD	Cannabidiol
C_{max}	Maximum plasma concentration
C_{trough}	Trough plasma concentration
CYP	Cytochrome P450
MCT	Medium-chain triglyceride
SAP	Systolic arterial blood pressure
$t_{1/2\lambda}$	Terminal half-life
t_{max}	Time to maximum plasma concentration

seed oil[10,a] or CBD and cannabidiolic acid (1:1) in an olive oil base[4] or flavored soft chew,[7] to the authors' knowledge, no studies have been reported regarding the pharmacokinetics of CBD following a period of repeated administration. Such information on CBD, particularly as a highly purified molecular entity, following chronic (repeated) and not just acute (single dose) administration would be important in establishing administration guidelines for its therapeutic use in veterinary species.

In light of the aforementioned evidence gaps, the objectives of the study reported here were 2-fold. First, we set out to determine the safety profile of 4 different doses of CBD (1, 2, 4, or 12 mg/kg, PO), as a single molecular entity, administered to dogs once daily for 28 days. Our second objective was to ascertain the effect of repeated administration of these doses on the pharmacokinetic profile of CBD and 2 metabolites, 7-COOH-CBD and 7-OH-CBD. To the authors' knowledge, this would be the first study of changes in the pharmacokinetics of CBD and 2 of its metabolites in dogs following chronic daily oral CBD administration.

Materials and Methods

Ethics statements

All animal care and experimental procedures were conducted under protocols approved by the Veterinary Drug Directorate, Health Canada (which issued an experimental study certificate), and the institutional animal care and use committee of the authors' research facility (VivoCore Inc; protocol No. VRI150-17175-CS). Study procedures were also performed in accordance with the Principles of the Animals for Research Act[11] and guidelines of the Canadian Council on Animal Care.[12]

Animals

Twenty-four healthy purpose-bred Beagles (age, 1 year 9 months; body weight, 8 to 15 kg) from a colony owned by the contract research facility were considered for inclusion in the study. Health status was assessed by a veterinarian. Dogs were excluded if they were pregnant or lactating, uncooperative, or receiving medications or supplements during the course of the study that could interfere with study objectives or had received cannabinoid agents within 30 days before the study began. Of the 24 dogs, 4 were excluded owing to an uncooperative disposition during handling (n = 2), use of medications for puncture wounds (1), or difficulty with group housing (1). Consequently, 20 dogs were ultimately included. Dogs were allowed 14 days to acclimate to the study environment before the study began.

Treatments

To obtain CBD for use in the study, *Cannabis sativa* plants were grown indoors under tightly controlled environmental conditions at Canopy Growth Corporation. The CBD from dried plant material was derived by supercritical CO_2 extraction. The extracted CBD was purified (> 95% CBD wt/wt, corrected for moisture) and diluted with MCT oil[b] to achieve 4 target concentrations of CBD: 7.5, 15, 30, and 90 mg/mL.

Cannabinoid analysis was performed by means of a validated high-performance liquid chromatography method with diode-array detection[c] and a reverse-phase C18 column[d] (mobile phases: 0.1% phosphoric acid in acetonitrile, methanol, 0.1% phosphoric acid in water, and water). Acceptance criteria for the molecular standards to achieve for the chromatographic method prior to quantitative analysis of the unknown dog plasma samples included accuracy (≤ 10% relative bias), repeatability (≤ 10% relative SD), and intermediate precision (≤ 20% relative SD). The lower level of quantification for CBD was 0.04% (wt/wt). Samples were tested in duplicate. Detected CBD concentrations (relative mean SD) were 7.27 mg/mL (0.9%), 14.96 mg/mL (0.4%), 29.97 mg/mL (0.7%), and 97.95 mg/mL (2.3%), all of which were within 10% of the expected target concentrations; these formulations were used to achieve daily CBD doses of 1, 2, 4, or 12 mg/kg, respectively.

For the CBD and placebo oils, concentrations of other cannabinoids were below the lower level of quantification (cannabinol, < 0.16% wt/wt; cannabichromene, < 0.13% wt/wt; Δ-8-tetrahydrocannabinol, < 0.18% wt/wt; Δ-9-tetrahydrocannabinol, < 0.02% wt/wt; Δ-9-tetrahydrocannabinol acid, < 0.06% wt/wt; cannabigerol, < 0.18% wt/wt; cannabigerolic acid, < 0.39% wt/wt; and cannabidiolic acid, < 0.04% wt/wt). The CBD formulations and placebo MCT oil[b] were stored at the study facility between 20.4°C and 24.5°C in a locked controlled drug cabinet protected from light. Prior to treatment, an opaque label was applied to cover the study products, thereby obscuring their identity.

Study design

A randomized, blinded, placebo-controlled trial was conducted. Dogs were first assigned to 5 sex-balanced treatment groups (n = 4 dogs/group) on the basis of the sum of ranks of their body weight. Then, the groups were randomly assigned by use of a random number generator[e] to a different treatment condition (CBD oil at 1, 2, 4, or 12 mg CBD/kg or placebo oil). Information regarding treatment allocation was securely stored and inaccessible to blinded research personnel.

After an approximately 12-hour food withholding period, administration of the assigned treatment began. Treatments (1 to 2 mL) were administered PO by syringe once daily for 28 days. All technicians administering the treatments were blinded to treatment allocation.

Animal housing and care

Dogs in the same treatment group were housed and exercised together (4 dogs/4.6 X 1.5-m pen). All

housing areas were cleaned and disinfected daily in accordance with standard operating procedures. Environmental controls for the housing areas were electronically set to maintain a temperature of 20.1°C to 25.2°C and a 12-hour light-to-dark cycle.

Dogs were fed a standard commercial dry dog food (≥ 26.0% crude protein, ≥ 16.0% crude fat, and ≤ 3.0% crude fiber)[f] once daily, at least 4 hours after treatment administration. The quantity of food offered was chosen to maintain body condition. Food was available for 1 hour, whereas water was available ad libitum. Animal health observations were conducted twice daily (in the morning before treatment administration and in the afternoon approx 6 hours after administration) by technicians who were blinded to treatment identity. Dogs were fitted with accelerometers[g] for the duration of the study to assess changes in motor activity. On completion of the study, dogs were returned to their colony.

Outcome assessment

The following outcomes were assessed at predetermined time points by a veterinarian or technicians unaware of treatment identity: animal health observations (twice daily during the 4-week treatment period and subsequent 1-week follow-up period); veterinary examination results (once during acclimation, at baseline [1 day before treatment began], and weekly during the treatment period); food consumption and 24-hour activity, measured daily beginning 2 weeks before treatment began through to the end of the treatment period; and body weight measured with a certified, calibrated scale twice during acclimation, at baseline, and weekly during the treatment and follow-up periods. The following outcomes were also assessed by a veterinarian blinded to treatment identity at baseline and after 2 and 4 weeks of treatment administration: intraocular pressure,[h] tear production (Schirmer tear test), SAP (Doppler method), and ECG parameters.[i]

Blood samples were collected for CBC and serum biochemical analysis twice during acclimation and weekly during the treatment and follow-up periods. Specifically, 4 mL of blood was drawn via 21-gauge needles by cephalic or jugular venipuncture; 2 mL was transferred to an evacuated serum separator tube and another 2 mL into an evacuated tube containing K_2-EDTA (ie, K_2-EDTA tube). Blood samples used to determine the 24-hour pharmacokinetic profile of CBD, 7-COOH-CBD, and 7-OH-CBD were collected before (ie, on day 1) and 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours after the first and last doses of the assigned treatments were administered. For this purpose, 2 mL of blood was drawn and transferred to an evacuated K_2-EDTA tube. For determination of 24-hour C_{trough} values, blood samples were collected 24 hours after the first dose of the assigned treatment was administered (ie, on day 2), midway through treatment (day 16), and following the last dose (day 29). Urine samples for urinalysis were collected by natural voiding (95% of collections), cystocentesis (3% of collections), or catheterization (2% of collections) at baseline and after 2 and 4 weeks of treatment administration.

Throughout the study, dogs were observed for any signs that would not be expected in clinically normal dogs. Any abnormality was considered an AE. The clinical importance of abnormal signs following treatment administration and changes in each measured outcome variable were evaluated by the attending veterinarian, who used clinical judgment to assess the presence and severity of AEs. Each AE was rated mild, moderate, or severe, depending on its impact on the dog's activities of daily living.[13]

Blood and urine sample handling and analysis

Urine samples were stored and refrigerated in sterile containers and shipped on ice packs on the day of collection for analysis at a reference laboratory.[j] Methods used for blood processing were previously described.[9] Blood samples in serum separator and K_2-EDTA tubes were stored at 2°C to 8°C and transported on the day of collection for analysis by standard laboratory methods[k] (CBC and serum biochemical analysis) or for further processing. For the K_2-EDTA samples intended for measurement of CBD and metabolite concentrations, plasma was separated into equal aliquots and stored at –80°C until shipment on dry ice to a bioanalytic laboratory[k] for analysis.

For measurement of plasma CBD, 7-COOH-CBD, and 7-OH-CBD concentrations, the analytic references were CBD,[l] 7-COOH-CBD, and 7-OH-CBD.[m] Internal standards were a deuterated analog of CBD (CBD-d_3) and paclitaxel (for 7-COOH-CBD and 7-OH-CBD) mixed in a 50:50 methanol-acetonitrile solution; 30 µL of this internal standard working solution was combined with 10 µL of standards or plasma samples. Samples were vortexed and centrifuged at 1,992 X g to concentrate precipitated plasma proteins to the bottom of the vial. Then, 25 µL of the supernatant was mixed with 25 µL of 0.1% formic acid in water, and 20 µL of this mixture was injected for analysis by means of qualified liquid chromatography–tandem mass spectrometry[n] and a phenyl-hexyl column.[o] Ten calibration standards in the range of 0.25 to 1,000 ng/mL (CBD), 0.5 to 1,000 ng/mL (7-COOH-CBD), or 1 to 1,000 ng/mL (7-OH-CBD) were used, including a blank sample (without internal standard) and zero sample (with internal standard). Acceptance criteria for method qualification and sample analysis included requirements that at least 67% of the nonzero calibration standards included in the calibration curve with all back-calculated concentrations be within a deviation of 20% from nominal concentrations (except for the lower level of quantification, where deviation within 25% was acceptable), the correlation coefficient (r) for the calibration curve be ≥ 0.99, and the area ratio variation between the prerun and postrun injections of the system suitability samples be within

25% of each other. Methodological and instrumentation details were previously described.[9]

Pharmacokinetic analysis

Pharmacokinetic parameters were estimated by noncompartmental methods.[p] For each animal, the area under the time-concentration curve and AUC_{0-last} (representing total systemic exposure) were calculated by the linear up–log down trapezoidal rule. The terminal rate constant (λ_z) was estimated by regression analysis of a minimum of 3 time points from the terminal (log-linear) portion of the concentration-versus-time curve. Values for $t_{1/2\lambda}$ were calculated as $\ln(2)/\lambda_z$. Values for t_{max} and C_{max} were determined from the nominal values.

Statistical analysis

Descriptive statistics were generated for all data.[q] Generalized estimating equations[14,15,r] were used to compare the CBC and serum biochemical data, pharmacokinetic parameters, CBD C_{trough}, body weight, ophthalmic variables (intraocular pressure and tear production), and SAP among treatment groups. For pharmacokinetic parameters and CBD C_{trough}, the 1-mg/kg group was used as the reference group; for the remaining variables, the placebo group served as the reference group. In all models, time was coded as a dummy variable, with the baseline assessment serving as the reference category so that all estimated parameters reflected a change in the outcome variable from baseline. Adjustment for false discovery rate inflation due to multiple comparisons was implemented within each model following the methods of Benjamini and Hochberg.[16] All tests were 2-tailed, and values of $P < 0.05$ were considered significant.

Results

Animals

Mean ± SD body weights of dogs in the placebo and 1-, 2-, 4-, and 12-mg/kg CBD groups (n = 4 dogs/group) at baseline (1 day before treatment began) were 11.5 ± 2.0 kg, 11.0 ± 2.3 kg, 11.5 ± 2.4 kg, 11.3 ± 2.5 kg, and 11.1 ± 0.7 kg, respectively. No significant difference in mean body weights was identified among the groups.

CBD safety and tolerability

Following a comprehensive assessment of the measured outcomes by the attending veterinarian, and in consideration of the diagnostic assessments to identify potential AEs (including medical history, physical examination findings, and CBC, urinalysis, and radiography results as dictated by the individual details of the AE), once-daily oral administration of CBD (in MCT oil) at doses of 1 to 12 mg/kg for 28 days was determined to be well tolerated by the dogs. No clinically relevant changes were observed related to the administration of any dose of CBD. Physical and neurologic examinations revealed no clinically important abnormal findings throughout the study.

Ophthalmologic examinations (intraocular pressure and Schirmer tear test; **Figure 1**) and SAP assessments (**Supplementary Figure S1**, available at: avmajournals.avma.org/doi/suppl/10.2460/ajvr.82.5.405) revealed no abnormal values during the 4-week treatment period that were considered clinically relevant by the attending veterinarian, nor was a dose-specific effect evident for CBD, as also indicated by the absence of clinically relevant differences in these findings between the CBD and
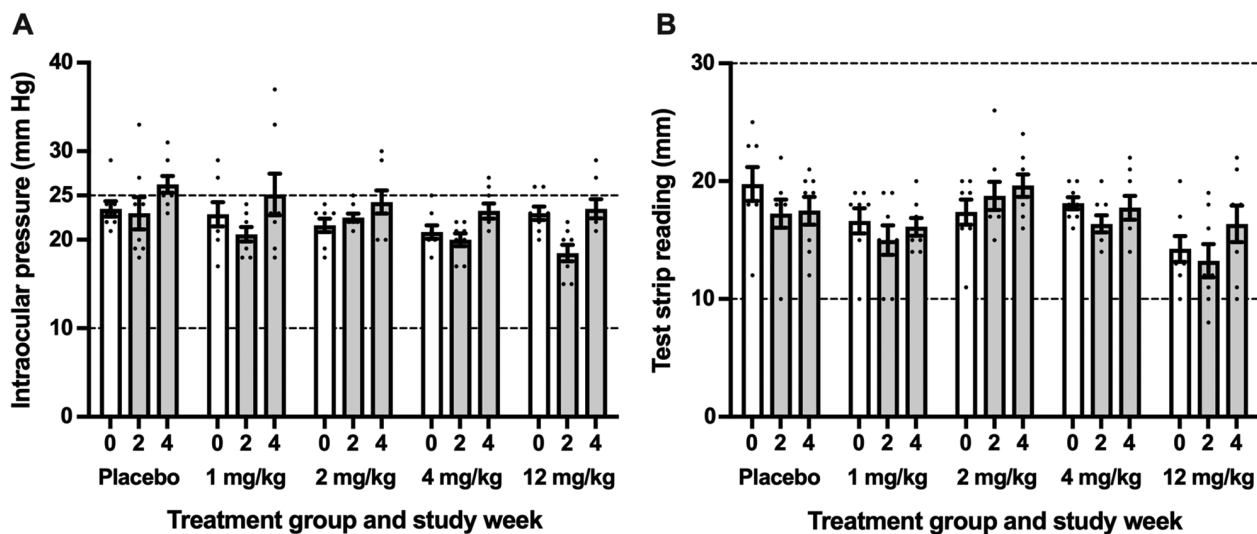

Figure 1—Mean (bars), SEM (error bars), and individual data points (dots) for intraocular pressure (A) and tear production (Schirmer tear test; B) in healthy adult Beagles (n = 4 dogs/group or 8 [1/eye] data points/group) at baseline (1 day before treatment began; week 0) and after 2 and 4 weeks of once-daily oral administration of a placebo (MCT oil) or CBD (in MCT oil; 1, 2, 4, or 12 mg/kg). Horizontal dashed lines depict the reference ranges for intraocular pressure (10 to 25 mm Hg) and Schirmer test strip readings (10 to 30 mm). No significant (ie, $P \geq 0.05$) differences were observed between the placebo group and the 4 CBD groups in mean changes in intraocular pressure and tear production from week 0 to week 4.
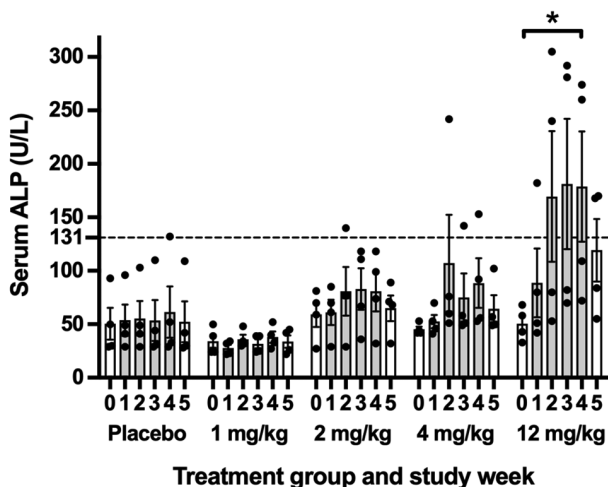
Figure 2—Mean (bars), SEM (error bars), and individual data points (dots) for serum ALP activity at various points for the dogs of Figure 1. The horizontal dashed line depicts the upper reference limit for serum ALP activity (131 U/L). *The mean change in serum ALP activity between 0 and 4 weeks differed significantly ($P = 0.03$) between the 12-mg/kg group and the placebo group. *See* Figure 1 for remainder of key.
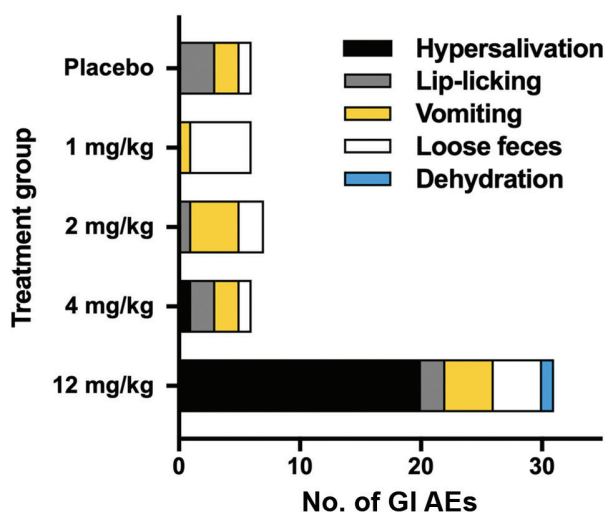

Figure 3—Total number of various types of gastrointestinal (GI) AEs observed during the 4-week treatment period for the dogs of Figure 1. For each dog in which an AE was observed on a given day, only the first occurrence of that type of AE was counted for that day (and not recurring episodes). *See* Figure 1 for remainder of key.

placebo groups. No clinically relevant findings were noted in ECG results for any dog throughout the study (data not shown). There were also no observable differences between groups in motor activity as assessed with an accelerometer during the course of the study.

No significant or clinically relevant changes in the 13 measured CBC variables occurred with CBD versus placebo administration (**Supplementary Tables S1 and S2**, available at: avmajournals.avma.org/doi/suppl/10.2460/ajvr.82.5.405). Changes in these

variables (22 parameters) were primarily minor (**Supplementary Table S3**, available at: avmajournals. avma.org/doi/suppl/10.2460/ajvr.82.5.405), with the most notable change occurring in serum ALP activity **(Figure 2)**. Although none of the 4 dogs in the 1-mg/kg group had a value that exceeded the upper reference limit (131 U/L), 1 of 4 dogs in each of the 2- and 4-mg/kg groups and 2 of 4 dogs in the 12-mg/kg group had values that exceeded this limit by up to 1.1-fold (2 mg/kg), 1.8-fold (4 mg/kg), or 2.3-fold (12 mg/kg), with increases detected following 1 week (12 mg/kg) or 2 weeks (2 and 4 mg/kg) of CBD administration. These increases in serum ALP activity generally began to decrease following 2 weeks of treatment in all 3 of these groups. In the 12-mg/kg group, the change from baseline to the end of the treatment period was significantly ($P = 0.03$) different from the change in the placebo group. Dogs with high serum ALP activity did not develop any associated clinical signs, and no concomitant increases were noted in other hepatic markers (ie, serum aspartate aminotransferase, alanine aminotransferase, and γ-glutamyl transpeptidase activities and total bilirubin concentration) during the treatment period.

Minor changes were noted in individual dogs with respect to urinalysis results (specific gravity, pH, protein, blood, WBC, RBC, crystals, epithelial cells, and bacteria; **Supplementary Table S4**, available at: avmajournals.avma.org/doi/suppl/10.2460/ajvr.82.5.405) that were considered clinically unimportant by the attending veterinarian on the basis of the specific parameter and in conjunction with CBC and serum biochemical results.

All AEs were mild and self-limiting, required no intervention, and occurred across all treatment groups, including the placebo group. No moderate or severe AEs were observed at any time. With respect to gastrointestinal AEs specifically, a comparable number of these AEs was noted in the 1-, 2-, and 4-mg/kg groups (n = 6, 7, and 6 AEs, respectively) and the placebo group (6; **Figure 3;** Supplementary Table S4). In contrast, the 12-mg/kg group had a greater number of gastrointestinal AEs (n = 31), with hypersalivation observed in 2 of 4 dogs, accounting for 65% (20/31) of those AEs. Hypersalivation in this group occurred both before (29% [9/31] of occurrences) and immediately after (71% [22/31] of occurrences) CBD was administered, resolving within 4 hours after administration. Nongastrointestinal AEs identified through daily animal observations or veterinary examinations were considered mild and nonserious and were noted across all treatment groups, including the placebo group (**Supplementary Tables S5 and S6**, available at: avmajournals.avma.org/doi/suppl/10.2460/ajvr.82.5.405).

Mild decreases in body weight were observed during the study. Mean ± SD body weights at the end of the 4-week treatment period for the placebo and 1-, 2-, 4-, and 12-mg/kg CBD groups were 11.4 ± 1.8 kg, 10.5 ± 2.3 kg, 10.8 ± 2.4 kg, 10.8 ± 2.5 kg, and 10.8

Table 1—Mean ± SD (range) values of pharmacokinetic parameters estimated for CBD after the first (day 1) and last (day 28) doses of CBD (in MCT oil; 1, 2, 4, or 12 mg/kg, PO) were administered to healthy adult Beagles (n = 4/group).

	1 mg/kg		2 mg/kg		4 mg/kg		12 mg/kg	
Parameter	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
AUC_{0-last} (ng•h/mL)	183 ± 43 (129–227)	288 ± 129 (96–377)	287 ± 178 (117–524)	959 ± 199*† (751–1,230)	859 ± 475† (446–1,530)	1,800 ± 597*† (997–2,420)	1,430 ± 610† (866–2,240)	2,890 ± 855*† (1,960–3,900)
C_{max} (ng/mL)	30 ± 7 (20–36)	53 ± 43 (6–110)	46 ± 23 (20–73)	115 ± 39ᵃ (68–163)	130 ± 47ᵇ (70–181)	194 ± 63 (106–249)	201 ± 55ᵇ (125–246)	285 ± 183 (150–539)
C_{max}/dose (kg•ng/mL/mg)	29.6 ± 6.9 (20.4–35.6)	53.3 ± 43.4 (6.1–110.0)	23.0 ± 11.5 (10.1–36.4)	57.5 ± 19.5* (33.9–81.5)	32.6 ± 11.9 (17.4–45.3)	48.5 ± 15.7 (26.5–62.3)	16.7 ± 4.6† (10.4–20.5)	23.8 ± 15.3 (12.5–44.9)
t_{max} (h)	4.5 ± 1.0 (4.0–6.0)	3.0 ± 0.8 (2.0–4.0)	3.5 ± 1.0 (2.0–4.0)	2.3 ± 0.5 (2.0–3.0)	3.5 ± 1.0 (2.0–4.0)	3.3 ± 0.5 (3.0–4.0)	4.5 ± 1.7 (3.0–6.0)	5.8 ± 4.5 (2.0–12.0)
$t_{1/2\lambda}$ (h)§	5.6 ± 1.0 (4.6–6.8)	24.6 ± 15.3 (6.9–43.3)	9.3 ± 6.6‡ (4.8–16.9)	19.0 ± 12.4 (8.3–36.8)	5.4 ± 1.4‡ (4.0–6.8)	22.7 ± 6.2‡ (15.6–26.9)	7.2‖ (NA)	13.8 ± 9.3‡ (3.7–21.9)
MRT_{0-inf} (h)§	7.9 ± 1.6 (6.0–9.7)	NA	11.9 ± 6.4‡ (7.1–19.2)	NA	8.0 ± 0.8‡ (7.4–8.9)	NA	10.4‖ (NA)	NA

*Within a group, mean value differs significantly ($P < 0.05$) between days 28 and 1. †Indicated mean differs significantly ($P < 0.05$) from that for the 1-mg/kg group at the same time point. ‡Values represent 3 dogs. §No statistical comparisons were performed owing to insufficient data. ‖Value represents 1 dog.

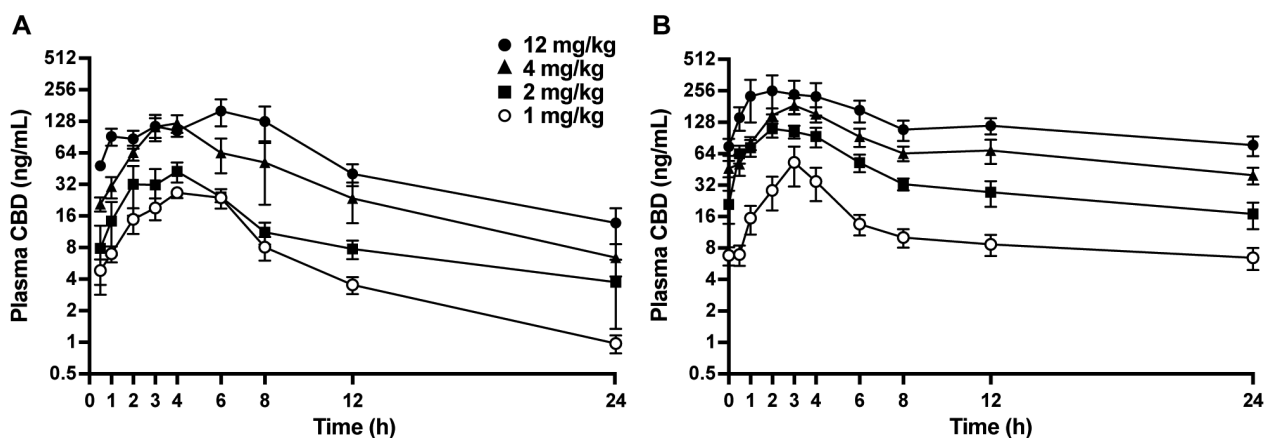MRT_{0-inf} = Mean residence time from time 0 to infinity. NA = Not applicable.

Figure 4—Mean plasma CBD concentrations in the dogs of Figure 1 at various points following administration of the first dose (A) and last treatment dose (B). Outliers were excluded from analysis. Data represent 3 dogs for the 1- and 2-mg/kg groups at 3 and 24 hours on day 1, 3 dogs for the 12-mg/kg group at 24 hours on days 1 and 28, and 4 dogs at all other data points. **See** Figure 1 for remainder of key.

± 0.8 kg, respectively, representing mean changes from baseline of –0.1 kg (–0.7%), –0.5 kg (–4.4%), –0.6 kg (–5.7%), –0.5 kg (–4.7%), and –0.3 kg (–2.3%), respectively (**Supplementary Figure S2**, available at: avmajournals.avma.org/doi/suppl/10.2460/ajvr.82.5.405). Compared with values in the placebo group, mean body weight reductions were significantly ($P < 0.01$) greater in the 1-, 2-, and 4-mg/kg groups, whereas differences in weight changes between the placebo and 12-mg/kg groups were not significant ($P = 0.39$). Although there appeared to be greater variability in daily food consumption in the CBD versus placebo groups during the dosing period, differences in variability were also present during the 14-day acclimation period (data not shown). Overall, food consumption was adequately maintained for each dog throughout the study.

Pharmacokinetics of CBD and metabolites

Mean total systemic exposure to CBD (AUC_{0-last}) increased in a dose-dependent manner following both acute (first dose) and chronic (28 days) CBD administration (**Table 1; Figure 4**). Following administration of the first CBD dose (1, 2, 4, or 12 mg/kg), mean AUC_{0-last} for CBD was 183, 287, 859, and 1,430 ng•h/mL, respectively. Values reached for CBD at 4 and 12 mg/kg were significantly ($P < 0.01$) higher (by 4.7- to 7.8-fold) than those reached at 1 mg/kg, with no significant difference between 1 and 2 mg/kg. At 28 days, mean AUC_{0-last} was 288, 959, 1,800, and 2,890 ng•h/mL, respectively. Values reached for CBD at 2, 4, and 12 mg/kg were significantly higher (by 3.3- to 10.0-fold) than those reached at 1 mg/kg. Within each CBD dose group, 28 days of CBD administration increased total systemic exposure to CBD by 1.6- to 3.3-fold, representing significant ($P < 0.01$) increases from first-dose values at 2, 4, and 12 mg/kg.

Whereas mean C_{max} increased in a dose-dependent manner following acute and chronic CBD administration, significant effects of dose on C_{max} were observed only for the first dose (Table 1). Peak CBD concentrations were significantly ($P < 0.01$) greater for CBD at 4 and 12 mg/kg (by 4.4- and 6.8-fold, respectively) than they were for CBD at 1 mg/kg, and no significant difference was noted between 1 and 2 mg/kg. However, C_{max} values normalized for dose (ie, C_{max}/dose) were comparable among 1, 2, and 4 mg/kg and significantly ($P < 0.01$) lower at 12 mg/kg.
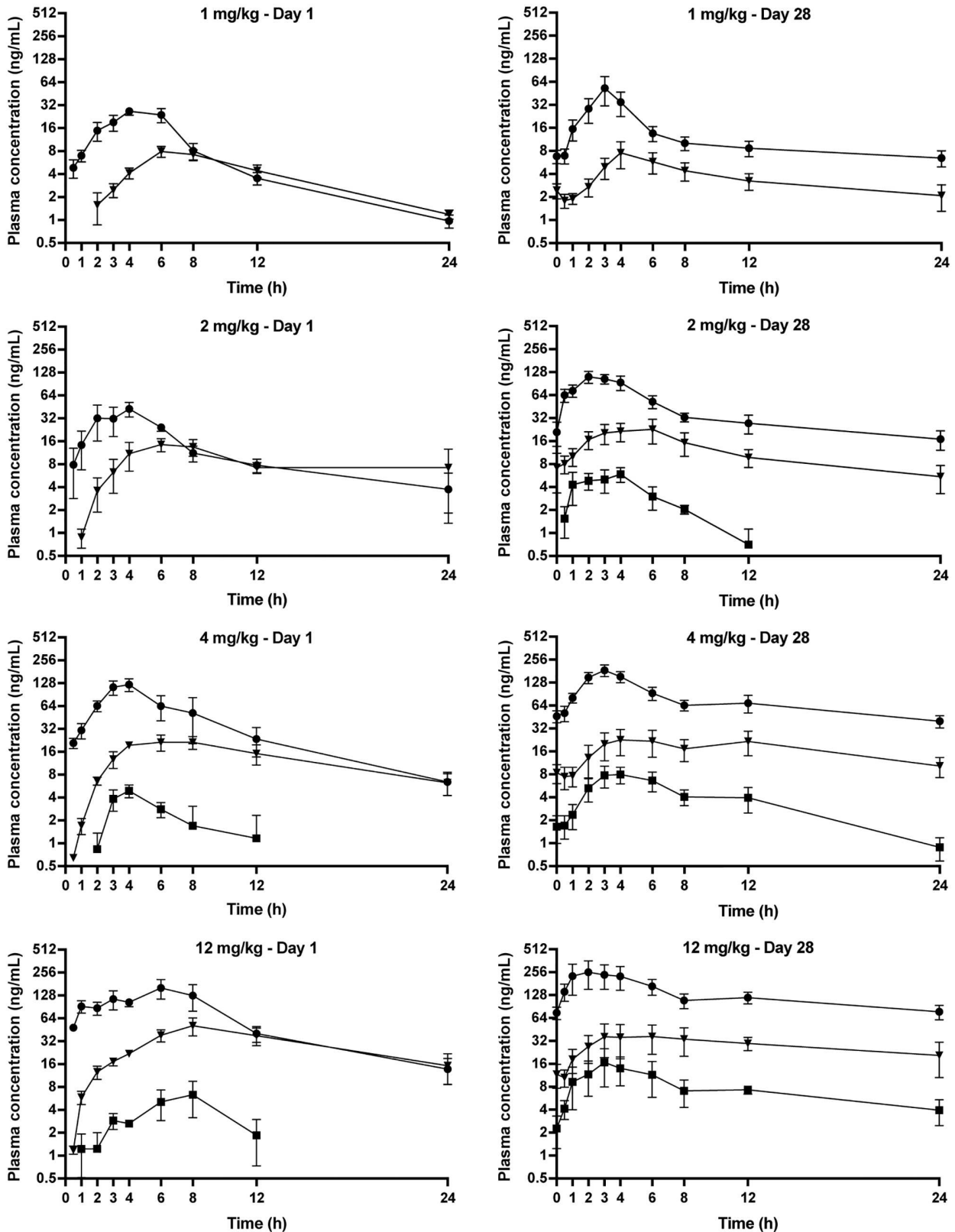
Figure 5—Mean plasma CBD (circles) and metabolite (7-COOH-CBD [triangles] and 7-OH-CBD [squares]) concentrations in the dogs of Figure 1 at various points. Note that the y-axis values were plotted on a log base 2 scale. Outliers were excluded from analysis. Data represent 3 dogs for the 1-, 2-, and 12-mg/kg groups at 3 and 24 hours on day 1, 3 dogs for the 12-mg/kg group at 24 hours on day 28, and 4 dogs at all other data points. Where no data are shown for 7-OH-CBD, concentrations were below the lower level of quantitation (1 ng/mL) for all dogs at most time points. *See* Figure 1 for remainder of key.

Table 2—Mean ± SD (range) values of pharmacokinetic parameters estimated for 7-COOH-CBD and 7-OH-CBD for the dogs of Table 1.

Parameter	1 mg/kg		2 mg/kg		4 mg/kg		12 mg/kg	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
7-COOH-CBD								
AUC_{0-last} (ng•h/mL)	86 ± 25 (52–106)	122 ± 107 (21–274)	169 ± 104 (70–279)	278 ± 141 (97–424)	307 ± 137† (185–446)	400 ± 248 (115–646)	610 ± 304† (199–862)	578 ± 203 (290–739)
C_{max} (ng/mL)	7.9 ± 2.6 (4.4–10.6)	10.9 ± 11.2 (1.1–27.0)	15.0 ± 6.3† (9.5–22.3)	24.2 ± 15.7 (6.5–44.8)	24.4 ± 8.5† (16.7–33.4)	27.6 ± 18.7 (6.2–46.4)	52.9 ± 24.7† (25.0–81.7)	46.6 ± 26.3 (20.9–83.4)
t_{max} (h)	6.0 ± 0.0 (NA)	11.5 ± 9.0 (4.0–12.0)	11.0 ± 8.7 (6.0–24.0)	4.3 ± 1.3 (3.0–6.0)	6.5 ± 1.9 (4.0–8.0)	5.8 ± 4.2 (3.0–12.0)	6.5 ± 1.9 (4.0–8.0)	9.8 ± 9.6 (3.0–24.0)
$t_{1/2\lambda}$ (h)	6.4 ± 1.1 (5.5–8.0)	13.8‖ (NA)	7.3‖ (NA)	10.6 ± 7.1* (5.7–18.7)	9.5 ± 1.1# (8.7–10.3)	13.5 ± 0.1# (13.4–13.6)	ND	9.5 ± 3.3* (6.1–12.6)
7-OH-CBD								
AUC_{0-last} (ng•h/mL)§	ND	ND	ND	34 ± 18 (17–53)	ND	84 ± 45 (26–124)	ND	157 ± 85 (77–248)
C_{max} (ng/mL)	1.3 ± 0.2 (1.1–1.6)	3.4 ± 2.2‡ (1.6–5.8)	2.2 ± 0.6†‡ (1.6–2.8)	6.6 ± 3.0* (3.7–10.1)	5.3 ± 2.1† (2.4–6.9)	9.2 ± 4.2 (3.8–14.0)	7.7 ± 4.9† (2.4–12.3)	17.9 ± 16.2 (5.3–40.8)
t_{max} (h)	6.0 ± 0.0 (NA)	3.3 ± 0.6* (3.0–4.0)	5.3 ± 2.3‡ (4.0–8.0)	3.0 ± 1.4 (1.0–4.0)	3.8 ± 0.5† (3.0–4.0)	3.5 ± 0.6† (3.0–4.0)	5.8 ± 2.6 (3.0–8.0)	5.5 ± 4.4 (3.0–12.0)
$t_{1/2\lambda}$ (h)§	ND	ND	ND	2.7 (NA)	ND	7.3 ± 2.0 (4.7–9.1)	ND	15.2 ± 16.2‡ (4.0–33.8)

#Value represents 2 dogs.
ND = Not defined.
See Table 1 for remainder of key.

Table 3—Mean ± SD C_{trough} (ng/mL) of CBD, 7-COOH-CBD, and 7-OH-CBD over the 4-week treatment period for the dogs of Table 1.

Analyte, by day	1 mg/kg	2 mg/kg	4 mg/kg	12 mg/kg
CBD				
Day 2	1.0 ± 0.3[a]	3.7 ± 4.2[a]‡	6.4 ± 4.4[a]	13.8 ± 8.9[a]
Day 16	4.7 ± 1.2[b]	16.4 ± 10.4[b]	55.5 ± 40.4¶,[b]	99.3 ± 49.4¶,[b]
Day 29	6.5 ± 3.1[b],‡	17.0 ± 9.7[b]	39.8 ± 14.4¶,[b]	77.3 ± 28.9‡¶,[b]
7-COOH-CBD				
Day 2	1.2 ± 0.3	7.2 ± 9.3‡	6.3 ± 4.0	15.3 ± 11.5‡
Day 16	ND	ND	10.7 ± 3.8	16.1 ± 8.3
Day 29	2.1 ± 1.4‡	5.5 ± 4.4	10.3 ± 6.0	20.7 ± 17.5‡
7-OH-CBD				
Day 2	ND	ND	ND	ND
Day 16	ND	ND	ND	6.9 ± 2.5
Day 29	ND	ND	1.2 ± 0.1	4.0 ± 2.5‡

¶The change from day 2 to day 16 or day 29 differs significantly ($P < 0.05$) from that for the 1-mg/kg group.
[a],[b]Within a column, mean values for CBD with different superscript letters indicate significant ($P < 0.05$) differences between time points. Statistic analyses were not conducted on the C_{trough} of metabolites because of insufficient data.
See Table 1 for remainder of key.

At day 28, normalized C_{max} values were comparable among all CBD dose groups. Within each dose group, C_{max} was generally unaffected by chronic administration, except when administered at 2 mg/kg.

Time to reach t_{max} was not significantly affected by dose after both acute and chronic administration of CBD. Following the first dose, mean t_{max} ranged from 3.5 to 4.5 hours, whereas following the last dose of CBD, it ranged from 2.3 to 5.8 hours (Table 1). Within each CBD dose group, chronic administration did not significantly affect mean t_{max} from values reached after the first dose (n = 4 dogs/group).

Although the elimination phase could not be defined for CBD in every dog that received it, mean $t_{1/2\lambda}$ ranged from 5.4 to 9.3 hours following the first dose (n = 11). Following chronic CBD administration, mean $t_{1/2\lambda}$ was notably longer, ranging from 13.8 to 24.6 hours (n = 14).

Of the 2 measured CBD metabolites (7-COOH-CBD and 7-OH-CBD), 7-COOH-CBD was more predominant **(Figure 5)**. Following the first dose, C_{max} of both metabolites increased in a dose-dependent manner, with a significantly ($P < 0.05$) higher C_{max} reached at 2, 4, and 12 mg/kg than at 1 mg/kg, but mean values were notably lower for 7-OH-CBD than for 7-COOH-CBD **(Table 2)**. Following repeated ad-

ministration of CBD for 28 days, the C_{max} of both metabolites no longer differed significantly among the CBD dose groups. Similarly, AUC_{0-last} for 7-COOH-CBD, which was significantly ($P < 0.01$) higher following the first CBD dose at 4 and 12 mg/kg than at 1 mg/kg, no longer differed significantly among the CBD dose groups following chronic administration.

For the 2 CBD metabolites, t_{max} was generally not dose dependent following both acute and chronic administration of CBD. Following the first dose, t_{max} for the metabolites ranged from 6.0 to 11.0 hours (7-COOH-CBD) and 3.8 to 6.0 hours (7-OH-CBD), and following chronic administration, it ranged from 4.3 to 11.5 hours and 3.0 to 5.5 hours, respectively (Table 2). Within each CBD dose group, chronic administration generally did not affect pharmacokinetic parameters for either metabolite, compared with values reached following the first dose.

Following 2 weeks of CBD administration (1, 2, 4, or 12 mg/kg), mean 24-hour C_{trough} of CBD was 4.7, 16.4, 55.5, and 99.3 ng/mL, respectively, representing significant ($P < 0.05$) 4.8-, 4.4-, 8.7-, and 7.2-fold increases, respectively, from values achieved following the first dose **(Table 3)**. For all CBD dose groups, mean 24-hour C_{trough} of CBD following 4 weeks of administration did not significantly differ from val-
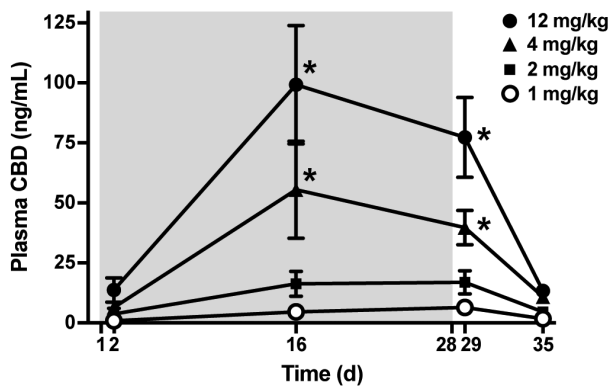
Figure 6—Mean values for 24-hour plasma CBD C_{trough} in the dogs of Figure 1 at the start of treatment (day 2), midway through treatment (day 16), end of treatment (day 29), and 1 week after treatment ended (day 35). The shaded area represents the treatment period. Outliers were excluded from the analyses. Data represent 3 dogs in the 1-, 2-, and 12-mg/kg groups on days 2 and 29 and 4 dogs for all other data points. *The mean change in CBD C_{trough} from days 2 to 16 or day 29 differed significantly ($P < 0.05$) from respective values for the 1-mg/kg group. **See** Figure 1 for remainder of key.

ues achieved at 2 weeks. A dose effect was observed: although no significant differences were identified between 1 and 2 mg/kg, mean C_{trough} of CBD was significantly ($P < 0.05$) higher at 4 and 12 mg/kg versus 1 mg/kg following both 2 and 4 weeks of CBD administration **(Figure 6)**. The mean ± SD accumulation index for plasma CBD concentration over the course of the 28-day treatment period was 2.1 ± 0.9 (1 mg/kg), 1.7 ± 0.7 (2 mg/kg), 1.8 ± 0.4 (4 mg/kg), and 1.5 ± 0.4 (12 mg/kg).

Discussion

The aim of the present study was to evaluate the safety and tolerability of chronically administered purified CBD over a fixed dose range and to evaluate changes in its pharmacokinetics following repeated administration, the latter of which had not yet been investigated in dogs. Findings indicated that once-daily oral administration of CBD in an MCT oil carrier at 1, 2, 4, or 12 mg/kg for 28 days was well tolerated by healthy Beagles. Veterinary examinations revealed no clinically important abnormal findings throughout the study, and no clinically relevant changes were noted in CBC, serum biochemical, intraocular pressure, tear production, SAP, ECG, or urinalysis data.

Other investigators have also shown CBD oil to be well tolerated by dogs when administered PO for 1 to 3 months at fixed dosages ranging from 0.5 to 1.2 mg/kg once daily[4] or 2 to 10 mg/kg twice daily (4 to 20 mg/kg/d)[5-8] or as 10 escalating doses (approx 2 to 62 mg/kg/dose).[9] In their critical review of the safety of CBD, the World Health Organization concluded that "CBD is generally well tolerated with a good safety profile . . . and relatively low toxicity."[17] Findings of the present study further corroborated these overall conclusions for dogs.

All treatment groups, including the placebo group, in the present study experienced AEs that were gastrointestinal in nature, albeit the frequency of these AEs was greatest in the 12-mg/kg group. The most common gastrointestinal AE in that group was hypersalivation, which occurred both before and immediately after treatment administration to 2 of 4 dogs. A previous placebo-controlled study[9] conducted by our research group to investigate the safety of serially administered CBD at higher doses (up to approx 62 mg/kg) similarly showed that hypersalivation is more common with a CBD-predominant oil versus an MCT oil placebo during and after administration. Taken together, these findings suggest that some dogs may have an aversion to chronic oral administration of higher CBD concentrations delivered in an unflavored MCT oil. In uncontrolled studies, loose feces has been noted to be a common AE following administration of CBD or CBD–cannabidiolic acid to unfed[7] and fed[5] dogs. In the present and previous[9] studies by our research group, wherein treatments were administered to unfed dogs, loose feces was not observed to be a major CBD-related AE because it occurred at similar frequencies between CBD and placebo (MCT oil) groups, suggesting that the MCT oil carrier may be contributing to mild gastrointestinal discomfort.

Analysis of all CBC and serum biochemical analytes evaluated in the present study indicated that the only abnormality was an increase in serum ALP activity that appeared to be dose related in some dogs. Such an increase in dogs receiving CBD has been observed by others[5,6,8,9] as early as 2 weeks following treatment initiation (10 or 20 mg/kg/d).[5] The increases in ALP values in the present study, observed as early as 1 week (12 mg/kg) or 2 weeks (2 and 4 mg/kg) after CBD administration began, changed to decreases following 2 weeks of administration in all 3 groups, suggesting an adaptive response. A 2012 Expert Workshop summary report prepared by the European Society of Toxicologic Pathology noted that increases in systemic ALP activity in the absence of hepatocellular degeneration in dogs could be interpreted as an adaptive rather than adverse response to drug exposure.[18]

In dogs, plasma or serum ALP activity can originate from the liver or bone or as a result of endogenous or exogenous corticosteroid hormones.[19,20] Alkaline phosphatase is also found in the intestines and kidneys, but these isoenzymes have brief half-lives and are thus not detected in plasma or serum.[19] Induction of hepatic drug-metabolizing enzymes is a plausible explanation for increases in plasma or serum ALP activity.[19] The liver is a major site of drug metabolism in mammals,[21] and hepatic CYP enzymes, particularly CYP1, CYP2, and CYP3, are considered to be the most important drug-metabolizing enzymes.[22] In dogs, drugs including phenobarbital have been shown to increase CYP, hepatic ALP (microsomal fraction), and serum ALP activities with no detectable hepatobiliary obstruction, bone damage, or clinical signs of liver disease.[23-27] Moreover,

findings from toxicological research involving dogs and monkeys in which induction of hepatic drug-metabolizing enzymes was confirmed suggest there is no consistent relationship between induction of those enzymes and hepatotoxicosis, as reflected in changes in clinical chemistry values.[20] Whereas CBD is metabolized by CYP, it also functions as an inhibitor of CYP (CYP1A, CYP2C, and CYP3A).[28] It is plausible that the increases in ALP activity observed with CBD are a result of CYP induction, as also proposed by other investigators of the effects of CBD in dogs.[6,8]

The finding that no dogs in the 1-mg/kg group and not all dogs in the 2-, 4-, and 12-mg/kg groups of the present study had an increase in serum ALP activity suggested that such increases may not be a hallmark of CBD administration and may be an adaptive response in only some dogs. Nonetheless, additional investigation is warranted into the effects of CBD on ALP activity, particularly to establish whether increases in activity are sustained over longer administration periods (eg, 3 to 6 months) or whether they normalize over time and are an acute adaptive response. The origin of any increases in serum ALP activity (ie, liver, bone, or corticosteroid hormones), the potential role of CYP induction in these increases, and whether these increases are associated with hepatobiliary insult also warrant further investigation.

A mild mean reduction in body weight was observed across all treatment groups in the present study, including the placebo group; however, a greater reduction was observed across the 4 CBD dose groups (2.3% to 5.7%), compared with the reduction in the placebo group (0.7%), that was not explained by changes in food consumption or daily activity or the sporadic occurrence of gastrointestinal AEs. Whereas MCT oil has been shown to affect body weight in rodents,[29] MCT oil was not a major constituent of the dogs' diets in the present study, and not all dogs in the placebo group had a body weight reduction. Nonetheless, it remains unclear whether the MCT oil contributed to mild variations in observed body weights. Interestingly, in rodents, coadministration of CBD with an antagonist of cannabinoid-2 receptors prevented the observed decreases in weight produced by CBD alone.[29] Also in rodents, oral administration of CBD (4.4 mg/kg) induced a significant reduction in total food consumption over a 4-hour test period, compared with that for vehicle-treated control animals[30]; however, when administered IP (3 to 100 mg/kg), no effects on food consumption were observed.[31,32] The effects of CBD on food consumption and body weight in dogs remain inconclusive and require further investigation.

Several differences were observed in pharmacokinetic parameters for CBD and selected metabolites following acute (first dose) versus chronic administration in the present study. Following chronic administration, total systemic exposure to CBD increased by approximately 2- to 3-fold from values reached after the first dose in all CBD dose groups. Moreover, as

a result of chronic administration, values for AUC_{0-last} increased in a dose-dependent manner, with systemic CBD exposure being 3.3-, 6.3-, and 10.0-fold higher with CBD administered at 2, 4, or 12 mg/kg versus 1 mg/kg, respectively. These differences may have been attributable to plasma accumulation over the study period. Indeed, the plasma accumulation index of CBD ranged from 1.5 to 2.1 across the 4 CBD doses. For all doses, the $t_{1/2\lambda}$ of CBD also increased following repeated administration, suggesting less rapid CBD excretion with plasma accumulation. Following chronic administration, dose-normalized values for C_{max} were similar for CBD across all CBD doses, suggesting the existence of linear kinetics for all doses including the highest dose (12 mg/mg), and plasma CBD concentrations increased proportionally to the administered dose. Regarding the selected metabolites (7-COOH-CBD and 7-OH-CBD), the significant dose-dependent effects on pharmacokinetic parameters (AUC_{0-last}, C_{max}, or both) following the first dose were no longer detected after chronic administration, although dose-dependent increases were apparent. Interindividual variability in the metabolism of cannabinoids is well-known[10,33] and likely explained by differences in the expression and function of CYP.[34] The higher variability in the pharmacokinetic parameters for CBD metabolites following chronic administration may explain the lack of significant differences.

Several studies have demonstrated the efficacy of CBD when repeatedly administered PO to dogs with painful osteoarthritis (1.2 to 2 mg/kg, twice daily for 4 weeks)[4,8] or epilepsy (2.5 mg/kg, twice daily for 12 weeks).[5] However, the relationship between blood CBD concentration following chronic administration and therapeutic response is largely unknown. The type and stage of disease, refractoriness to past drug exposure, comorbidities, involvement of current treatments, and age and breed of the dog are all important when considering the starting dose and dose adjustment. Additional studies will be needed to elucidate whether CBD dose adjustment may be effective in partially responsive or nonresponsive veterinary patients.

Although the efficacy of CBD was not evaluated in the present study, information was obtained on the relationship between CBD dose when administered PO for 28 days and a sustained plasma CBD concentration over time. Values for the 24-hour C_{trough} of CBD were dose dependent; chronic administration yielded maximum individual values of approximately 10 ng/mL (1 mg/kg), 20 ng/mL (2 mg/kg), 50 ng/mL (4 mg/kg), and 100 ng/mL (12 mg/kg). Importantly, within each dose group, mean values for C_{trough} of CBD were comparable after 2 and 4 weeks of administration, indicating that a steady-state plasma concentration had occurred within approximately 2 weeks after administration began.

A strength of the study reported here was that we evaluated the safety and pharmacokinetics of

chronically administered CBD in an oil matrix that, along with tinctures, is a formulation that is easily amenable to dose adjustment.[35] Other formulations of CBD can be purchased for dogs, such as soft chews, soft gels, tablets, and creams, and additional research is warranted into their safety and pharmacokinetic profiles following chronic administration.

Limitations of the present study should be acknowledged and included the small size of each treatment group (n = 4), particularly given the observed interindividual variability in pharmacokinetic parameters for CBD and its metabolites. During statistical analysis, correction for multiple comparisons was applied within variables but not across variables, which may have inflated the rate of false-positive results (ie, type 1 error). Given that the terminal pharmacokinetic phase was not well-defined for every dog over the 24-hour blood collection period, the time to achieve a steady state and the relationship between dose and elimination half-life were challenging to determine. Our findings suggested that future pharmacokinetic studies should incorporate additional blood collection time points that also span a longer period (ie, > 24 hours).

Overall, chronic administration of a highly purified CBD isolate in MCT oil (1 to 12 mg/kg, PO, once daily for 28 days) in the present study was well tolerated by healthy adult Beagles, and no clinically relevant changes in measured safety outcomes were observed at any point. The observed increases in serum ALP activity, which may have represented an early adaptive response to CBD metabolism, warrant further investigation in longer-term studies. Repeated administration led to CBD accumulation in circulation and dose-dependent increases in total systemic exposure and 24-hour C_{trough} of CBD. The observed maximum individual C_{trough} values for CBD following chronic oral administration (approx 10, 20, 50, and 100 ng/mL for 1, 2, 4, and 12 mg/kg/d, respectively) may guide selection of a starting dose or dose adjustment of a comparable formulation for therapeutic indications. Additional research is needed to establish the therapeutic effectiveness of a given plasma CBD concentration when CBD is used as a primary or adjunctive treatment.

Acknowledgments

Footnotes

a. McGrath S, Colorado State University, Fort Collins, Colo: Personal communication, 2020.
b. Musim Mas, Singapore City, Republic of Singapore.
c. 1260 Infinity series G1315D diode array detector, Agilent Technologies Inc, Santa Clara, Calif.
d. Poroshell 120-EC-C18 column, Agilent Technologies Inc, Santa Clara, Calif.
e. Excel 2016, Microsoft Corp, Redmond, Wash.
f. Purina ProPlan Savor Adult Chicken and Rice Formula, Nestlé Purina PetCare Co, St Louis, Mo.
g. Actical device, Philips Respironics, Montreal, QC, Canada.
h. Icare Tonovet Plus tonometer, Tonovet, Vantaa, Finland.
i. ECG transmitter with limb plate electrodes, Idexx Laboratories Inc, Markham, ON, Canada.
j. Antech Diagnostics, Mississauga, ON, Canada.
k. InterVivo Solutions Inc, Mississauga, ON, Canada.
l. Sigma-Aldrich Co, St Louis, Mo.
m. Toronto Research Chemicals Inc, Toronto, ON, Canada.
n. QTRAP 6500 LC-MS/MS System, Sciex, Framingham, Mass.
o. Kinetex phenyl-hexyl column (2.1 X 50 mm; 2.6 μm), Phenomenex Inc, Torrance, Calif.
p. Phoenix WinNonlin, version 8.0, Certara, Mountainview, Calif.
q. Prism, version 8.1.2 for Windows, GraphPad Software, San Diego, Calif.
r. R, version 4.0.0, R Foundation for Statistical Computing, R Core Team, Vienna, Austria.

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