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SHORT COMMUNICATION

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Serum cannabidiol, tetrahydrocannabinol (THC), and their native acid derivatives after transdermal application of a low-THC *Cannabis sativa* extract in beagles

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Abstract

Cannabinoids hold promise for treating health problems related to inflammation and chronic pain in dogs, in particular cannabidiol (CBD), and its native acid derivative cannabidiolic acid (CBDA). Information regarding systemic delivery of cannabinoids through transdermal routes is sparse. The purpose of this study was to determine pharmacokinetics of transdermal administration of a low-THC *Cannabis sativa* extract in healthy dogs. Six purpose-bred research beagles were treated with a transdermal CBD-CBDA-rich extract, and serum concentrations of CBD, CBDA, tetrahydrocannabinol (THC), and its acid derivative tetrahydrocannabinolic acid (THCA) were examined prior to and at the end of weeks 1 and 2. A 4 mg/kg dose of total cannabinoids twice daily resulted in appx 10 ng/ml of CBD, 21–32 ng/ml of CBDA, trace amounts of THCA, and unquantifiable amounts of THC in serum at the end of weeks 1 and 2 of treatment. Results showed that CBDA and THCA were absorbed better systemically than CBD or THC.

KEYWORDS

cannabidiol, cannabidiolic acid, dog, hemp, tetrahydrocannabinol, transdermal

Cannabinoids have been identified and used in both human and animal medicine for conditions including osteoarthritis, neuropathies, seizure disorders, and skin disease (Gamble et al., 2018; Landa, Sulcova, & Gbelec, 2016; McGrath, Bartner, Rao, Packer, & Gustafson, 2019; White, 2019). Presently, low-THC *Cannabis sativa* (i.e., hemp) cannabidiol (CBD)-rich products used orally have shown some efficacy for arthritis and seizures in dogs (Gamble et al., 2018; McGrath et al., 2019), the range of cannabinoids being delivered include small amounts of Δ9-tetrahydrocannabinol (THC) and the acid derivatives of these products (cannabidiolic acid [CBDA] and THCA) and other minor cannabinoids; all in compliance with regulatory standards for "hemp" being below 0.3% THC and its acid derivative.

Although oral consumption of oils or edible forms of hemp extract is used across species (Birnbaum et al., 2019; Gamble et al., 2018; McGrath et al., 2019; Taylor, Gidal, Blakey, Tayo, & Morrison, 2018), in veterinary medicine transdermal approaches are sought to ease delivery and prevent negative relationships between the owner and animal. There are limited data on the use of transdermal delivery of CBD and THC; however, preclinical models and human data suggest ample delivery of CBD and/or THC can be achieved (Bruni et al., 2018; Hammell et al., 2016; Hu, Cullen, Tang, & Fang, 2020; Lodzki et al., 2003; Niteckta-Buchta et al., 2019). Recently, a publication in dogs suggested that steady-state serum concentration of CBD is around 100–200 ng/ml when providing 10–20 mg/kg of an undisclosed transdermal form of CBD-rich hemp extract twice daily, suggesting dermal absorption of CBD (Bartner, McGrath, Rao, Hyatt, & Wittenburg, 2018); however, aural erythema was a common side effect of the treatment which may be due to the ointment formulation (McGrath, Bartner, Rao, Kogan, & Hellyer, 2018). It is currently unknown how effective transdermal approaches are for the delivery of the native acid forms of CBD and THC; CBDA and THCA, respectively.

The objective of our study was to use a low-THC *Cannabis sativa* extract compounded in the transdermal formulation that contained equal amounts of CBD and CBDA with lesser amounts of THC and THCA (30:1 ratio) to determine serum concentration of CBD, CBDA, THC, and THCA utilizing this form of transdermal delivery twice daily for two weeks in dogs.

Six purpose-bred female research beagles between the ages of 18–36 months weighing between 8.2 and 9.8 kg were treated with approximately 4 mg/kg of low-THC *Cannabis sativa* based ethanol extract that was emulsified with Pencream (HUMco) base in a 1:7.5 ratio producing a compounded ointment with a final concentration of 32 mg/ml CBD, 33 mg/ml CBDA, 1.3 mg/ml THC, and 1.0 mg/ml THCA (Ellevet Sciences) based on analysis at the beginning and at the end of experimentation. The compounded transdermal hemp extract was delivered in 0.1 cc increments onto the pinna of the ear (approximately 0.6 cc per treatment) and rubbed into the area along the outer pinnae at 7 a.m. and 6 p.m. each day. Each dog was singly housed other than two enrichment times per day to prevent interactions between the dogs that would allow grooming. Examination of the dogs was performed by an animal technician daily and veterinary assessment twice weekly for any signs of somnolence, ataxia, or other potential systemic side effects from the applied transdermal formulation. At the end of the first week of treatment, three dogs showed some redness along the internal portion of the ear canal due to ointment migration into the canal; therefore, these three dogs had the remaining week of morning doses applied to one pinnae and the second dose in the evening was applied to the inner inguinal area, and dogs were watched during enrichment to ensure they did not disturb the area of delivery in the evenings.

Eight milliliter of blood was collected prior to the study (Background; week 0), week 1 and 2 (6 hr after morning transdermal application) in plain coagulation tubes and allowed to coagulate for 30 min before centrifugation. Samples were centrifuged at 3,600 *g* for 10 min. Serum was collected and immediately frozen at −80°C before sending to the laboratory for analysis.

Cannabinoids analysis was performed by a method allowing for simultaneously analysis of 10 cannabinoids and their metabolites at the Toxicology Research Laboratory, University of Illinois at Chicago. Reference standards for CBD and CBDA were obtained from Restek Corporation; all other reference and internal standards mentioned below were obtained from Cerilliant Corporation. The concentration of cannabinoids (CBD, CBDA, THC, and THCA) in dog serum was determined by high-performance liquid chromatography and tandem mass spectrometry (Nexera X2 and LCMS 8050; Shimadzu Corp.).

In short, dog serum (40 μl) was deproteinated and solvent extracted to produce supernatants appropriate for analysis. The processed samples were injected (10 μl) into Waters Atlantis T3 HPLC column (3 μm 2.1 × 50 mm) coupled to LC-MS/MS. The column was equilibrated with mobile phase A (0.1% formic acid in water) and mobile phase B (acetonitrile) at ratio A: B 50:50 for 0.5 min. The compounds were eluted by a linear gradient from 50% B to 100% B over 6 min and then hold 100% B for 1 min. Subsequently, the column

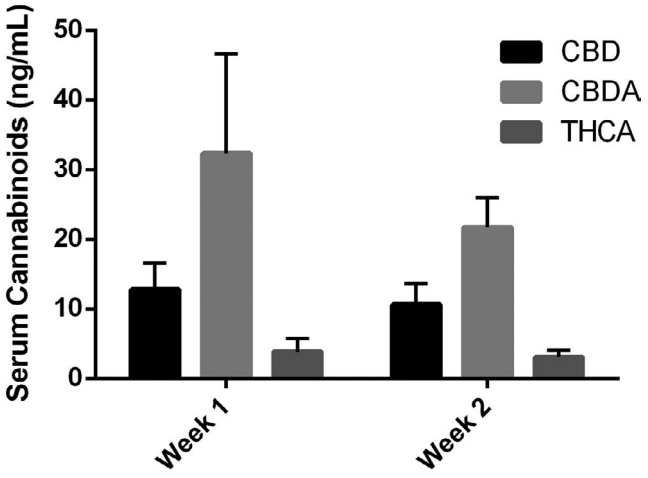

FIGURE 1 Mean and *SD*s for serum cannabinoid concentrations after twice daily transdermal application of hemp extract infused ointment to dogs ($n = 6$). No significant differences were noted between weeks for CBD, CBDA, or THCA

was re-equilibrated at initial composition for 0.5 min. Flow rate was 0.3 ml/min. Autosampler and column temperature were set at 4 and 30°C, respectively. CBD, CBDA, THC, and THCA were detected in ESI-positive mode using MRM transitions *m/z* 314.90 > 193.10, 359.10 > 219.10, 315.00 > 193.10, and 359.00 > 219.05, at retention time of approximately 4.3, 4.0, 5.3, and 5.8 min, respectively. Concentrations of cannabinoids were calculated by LabSolutions software (Shimadzu Corp.) using a quadratic calibration curve with $1/c^2$ weighing based on relative response (peak area of cannabinoids/peak area of internal standards). The calibration curve range was from 1 to 1,000 ng/ml for CBD, CBDA, THC, and THCA in dog serum.

Statistical analysis was performed for week 1 and 2 cannabinoid data using a Wilcoxon signed rank test due to the small population size. The *p* value for significance was set at $p < .05$ for all analyses.

No cannabinoids were detected in the dogs prior to the experimental regimen. Six hours after application of the transdermal formulation, the mean serum CBD concentration was (mean ± *SD*) 12.8 ± 3.8 and 10.6 ± 3.1 ng/ml at the end of week 1 and 2, respectively. At week 1 and 2, the serum concentrations of CBDA were 32.4 ± 14.3 and 21.7 ± 4.3 ng/ml, respectively. The mean serum concentrations of THCA were 3.8 ± 1.9 ng/ml at the week 1 and 3.1 ± 1.0 ng/ml at week 2 (Figure 1). Differences between week 1 and 2 cannabinoid concentrations were not statistically significant for any of the cannabinoids examined. THC concentrations were below the lower limit of quantitation (1 ng/ml) at weeks 1 and 2.

The use of transdermal applications of hemp-derived cannabinoids has been proven to be effective in clinical and preclinical models and human studies in temporomandibular joint disease and peripheral neuropathic pain (Hu et al., 2020; Niteckta-Buchta et al., 2019). The exact mechanisms of action related to CBD and associated cannabinoids effects on pain and whether these effects are through systemic or local effects on pain are unclear; however, similar findings related to mitigation of pain have been observed in preclinical rodent models examining the effects of cannabidiol on

models of osteoarthritis and dermatitis (Bruni et al., 2018; Hammell et al., 2016; Lodzki et al., 2003).

The transdermal application of cannabidiol or hemp oils, ointments, or creams with and without permeability enhancers such as endosomes, lipophilic substances, and solvents has been examined in many formats at different concentrations through either single application or patch like formulations making it difficult to compare effects (Bartner et al., 2018; Bruni et al., 2018; Paudel, Hammell, Agu, Valiveti, & Stichcomb, 2010). Few studies in vivo have examined serum concentrations during application since many clinical studies have focused on local pathology remediation. In one preclinical study, examining application of CBD to rodents after a single daily application of a complex gel system for 4 days on the back (after shaving) showed that transdermal application of 6.2 mg to rats delivered a large enough systemic dose to ameliorate Freuds adjuvant-induced arthritis and to achieve an average plasma concentration of 33 ± 10 ng/ml (Lodzki et al., 2003). Our 4 mg/kg dose of total cannabinoids twice daily resulted in approximately 10 ng/ml concentration in serum suggesting that higher doses may be necessary to achieve therapeutic doses of CBD systemically. It must also be noted that these transdermal applications are far from ideal as Bartner and colleagues showed that aural erythema was a side effect of the transdermal ointment used in their study, and similarly, we experience similar findings in some of our study dogs (Bartner et al., 2018). Whether this is due to residual terpenes from the hemp extract which when oxidized are known to cause dermal sensitivities or the ethanol extracts used in compounding require further elucidation (Bråred Christensson et al., 2016; Mills, Magnusson, & Cross, 2004).

Although our dosing may be slightly low for therapeutic systemic CBD delivery, it also revealed a CBDA concentration between 21 and 32 ng/ml. CBDA is the native form of CBD found in low-THC *Cannabis sativa* varieties which is often converted to CBD during heat extraction processes (Eichler et al., 2012). Alteration or degradation of the cannabinoids in the cream base after compounding was not an issue as analysis before and after experimentation was virtually identical (data not shown). Our data suggest CBDA absorption and retention is superior to CBD pending no unknown metabolic processes causing interconversion systemically. This is not surprising as oral dosing appears to show CBDA absorption across the gastrointestinal tract is superior to CBD (Pellise et al., 2018). To further support the idea that acidic cannabinoids show superior absorption and retention is the fact that THCA was also found in the serum with very low delivery concentrations (<1 mg/kg twice daily), and THC which was delivered at a similar concentration, but could not be found in the serum of dogs in this study. This may be an important point since CBDA has been shown to be an anti-inflammatory with direct cyclooxygenase inhibiting capabilities in cell culture and preclinical models, unlike CBD (Rock, Limebeer, & Parker, 2018; Takeda, Misawa, Yamamoto, & Watanabe, 2008), potentially making CBDA a better choice for clinical study of local and systemic disease using transdermal approaches in veterinary medicine.

The use of transdermal CBD-rich low-THC *Cannabis sativa* products appears to have utility in treating systemic and local pathologies, although the clinical application is not well studied currently. Optimization of delivery systems whether through oils, ointments, creams, and permeation enhancers, as well as timing of application and location of application need to be examined. The bypassing of hepatic metabolism is an attractive means of systemic delivery of CBD; however, the findings of superior absorption of CBDA suggest that the acidic forms of cannabinoids may be a better focus for anti-inflammatory actions.

CONFLICT OF INTEREST

JJW is a consultant for Ellevet Sciences.

AUTHOR CONTRIBUTION

All authors have reviewed and approved this manuscript. KD, BNT, and MBH were involved in the data acquisition and analysis. JJW, LJG, and AZ were involved in the project design and data analysis. AZ, AI, and AL were involved in the sample analysis and data acquisition.

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