REVIEW ARTICLE
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Cannabis sativa in veterinary medicine: Foundations and therapeutic applications

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ABSTRACT

An increase in products containing phytocannabinoids, particularly cannabidiol, is often observed in human and veterinary markets following the legalization of hemp (cannabis) for industrial purposes. In veterinary medicine, derivatives of *Cannabis sativa* are used for managing pain (osteoarticular, oncological, and neuropathic), epilepsy, and behavioral disorders, as well as oncological, immune-mediated, cardiovascular, and respiratory diseases. In addition, there is growing interest in incorporating *C. sativa* into livestock feed. To elucidate the mechanisms of action of phytocannabinoids, a thorough understanding of the endocannabinoid system and its role in maintaining homeostasis is essential. Short-term use of phytocannabinoid products appears generally safe, but further research is required to understand the routes of administration, pharmacokinetics, and pharmacodynamics across various species. Although literature on phytocannabinoids in veterinary patients is limited, the available data suggest significant therapeutic potential.

RÉSUMÉ

Cannabis sativa en médecine vétérinaire : fondements et applications thérapeutiques

Une augmentation des produits contenant des phytocannabinoïdes, notamment du cannabidiol, est souvent observée sur les marchés humains et vétérinaires à la suite de la légalisation du chanvre (cannabis) à des fins industrielles. En médecine vétérinaire, les dérivés du *Cannabis sativa* sont utilisés pour gérer la douleur (ostéoarticulaire, oncologique et neuropathique), l'épilepsie et les troubles du comportement, ainsi que les maladies oncologiques, immunitaires, cardiovasculaires et respiratoires. En outre, l'incorporation de *C. sativa* dans l'alimentation du bétail suscite un intérêt croissant. Pour élucider les mécanismes d'action des phytocannabinoïdes, une compréhension approfondie du système endocannabinoïde et de son rôle dans le maintien de l'homéostasie est essentielle. L'utilisation à court terme de produits phytocannabinoïdes semble généralement sécuritaire, mais des recherches supplémentaires sont nécessaires pour comprendre les voies d'administration, la pharmacocinétique et la pharmacodynamique chez diverses espèces. Bien que la littérature sur les phytocannabinoïdes chez les patients vétérinaires soit limitée, les données disponibles suggèrent un potentiel thérapeutique important.

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INTRODUCTION

Although medicinal use of products derived from the *Cannabis sativa* plant in animals is illegal in most countries, and reliable scientific literature is scarce, there is considerable interest in and demand for phytocannabinoid products. The increase in *C. sativa*-based products is due to the Farm Bill, a United States federal law passed in December 2018 that legalized production of hemp for industrial purposes, including supplements from the seed and plant, plus fiber production (1). In Canada, the Cannabis Act (2018) classifies all cannabinoids derived from the cannabis plant, including cannabidiol (CBD) and Δ-9-tetrahydrocannabinol (THC), as "cannabis." Availability of cannabis-containing products is more restricted for animals than for humans, as the Regulations on Access to Cannabis for Medical Purposes specifically refer to "persons," thereby excluding animals (2,3).

There is no established legal framework in Canada that allows veterinarians to prescribe cannabis-based medications for animals or permits manufacture and marketing of cannabis-based products (including pet food) specifically for pets. However, the Canadian Veterinary Medical Association and the Canadian Association of Veterinary Cannabinoid Medicine continue to advocate for cannabinoids in veterinary medicine and have provided evidence and advice to Health Canada (2). In addition to pharmaceutical products, companies will be able to produce Veterinary Health Products containing cannabis ingredients. This will be subject to compliance with requirements of the Veterinary Health Product Notification Program and inclusion of cannabis as a sanctioned ingredient in the List of Permitted Substances (2,4).

Hemp is a variety of *C. sativa* that is low in THC, a psychoactive molecule with intoxicating potential. However, the plant is rich in cannabidiolic acid (CBDA) and Δ-9-tetrahydrocannabinolic acid (THCA), which are nonintoxicating. During extraction, these compounds are decarboxylated to form THC and CBD, which are also psychoactive, but without psychotropic effects; *i.e.,* nonintoxicating. This prompted production of various hemp-based products such as oils, extracts, seeds, and fiber (4,5).

Interest for medicinal use of *C. sativa* has increased for both human and veterinary patients. This is due in part to increased access to CBD-rich extracts and changes in social perceptions and paradigms about the plant and the appeal of its potential therapeutic actions, coupled with regulatory changes in many countries (6).

In veterinary medicine, *C. sativa* derivatives are used for pain control (including osteoarticular, oncological, and neuropathic pain) and treatment of epilepsy, behavioral changes, and oncological, immune-mediated, inflammatory, cardiovascular, and respiratory diseases (4). Furthermore, there is interest in feeding it to livestock (7). Unlike in human medicine, there is a great paucity of data on *C. sativa* for therapies in animals; however, available studies imply much therapeutic potential (4–6).

HISTORY OF *CANNABIS SATIVA*

Cannabis sativa (cannabis) is an annual angiosperm, dioecious, herbaceous plant of the *Cannabaceae* family that likely originated in central Asia (India and China). One of the oldest cultivated plants, it was a source of fiber in China ~12 000 y ago, but its application has been controversial (4).

Cannabis *(C. sativa)* for medicinal purposes is not recent. The ancient Chinese Pharmacopoeia (the *Pen Ts'ao Ching*), written in the 3rd century BCE, described cannabis as a medicinal plant to treat a wide variety of diseases and symptoms, such as pain, inflammation, and some mental disorders (4).

Despite various ethnobotanical references, knowledge on cannabis was very limited until the 19th century, when the Irish physician William Brooke O'Shaughnessy began research in India in 1839, including clinical trials on rats, rabbits, cats, dogs, and horses for treating rheumatism, convulsions, cholera, tetanus, and hydrophobia, and reported that a cannabis extract was an effective antispasmodic. Cannabis for medicinal or recreational purposes was common in Europe and the United States between 1850 and 1930 and was sold in pharmacies for analgesic purposes (5). The first study on the use of cannabis (THC-rich cannabis, *Cannabis indica*) in domestic animals (dogs) by the physician and pharmacist Walter E. Dixon was published in 1899 (4).

In the last century, *C. sativa* as a medicine fell into disuse in many countries due to drug prohibition policies. For example, the Controlled Substances Act of 1970 included *C. sativa* (more precisely, marijuana, one of many plants in the genus *Cannabis* with high THC content) in the list of Schedule I category substances, along with heroin and lysergic acid diethylamide (LSD). Warnings of "high potential to cause dependence" and inappropriateness for medicinal purposes delayed research into therapeutic potential. Many countries are beginning to legalize cannabis for medicinal purposes and there is increasing research to support its use (5).

CHEMICAL AND PHARMACOLOGICAL CHARACTERISTICS OF *CANNABIS SATIVA*

Cannabis sativa comprises mainly cannabinoids (phytocannabinoids), terpenoids, and flavonoids. More than 554 components have been identified, including 113 phytocannabinoid compounds; the 2 most well-known are THCA and CBDA, the acidic and nonintoxicating forms before decarboxylation into THC and CBD. There are also 120 terpenoid compounds, volatile unsaturated hydrocarbons giving cannabis its unique smell (3,8,9), plus > 20 flavonoids that pigment the leaves and flowers, regulate cell growth, and attract pollinators. The 2 predominant flavonoids are canaflavin A and canaflavin B, with greater anti-inflammatory potential than aspirin. Other molecules include fatty acids, vitamins, minerals, proteins, and carbohydrates (5,8).

Studies are being conducted using THC and CBD, the 2 main phytocannabinoids in plant extracts, present in their acid form and requiring thermal conversion into the non-acid form (8). Cannabidiol was one of the first phytocannabinoids isolated (1940), though it was mistakenly considered inactive. In 1963, the stereochemical structure of CBD was discovered. Since then, research focused on THC, as it was assumed CBD was a non-active precursor to THC. In the 1970s and 1980s, CBD was shown to be effective in treating seizures, prompting research on CBD for medical use (3,10).

Preclinical studies, particularly in rodents, suggested CBD has potential for a wide range of conditions, including epilepsy, pain, anxiety, psychosis, and addiction. However, many studies used intraperitoneal injection *versus* oral administration, which may affect efficacy for certain conditions; *e.g.,* inflammation and cancer (3). Some human studies with CBD investigated epilepsy, pain, anxiety disorder, schizophrenia, and addiction. The fact that CBD has high efficacy in relieving seizures in children with Dravet syndrome, Lennox-Gastaut syndrome, and complex tuberous sclerosis has enhanced acceptance (3,10,11).

THE ENDOCANNABINOID SYSTEM

The endocannabinoid system (ECS), a regulatory system to maintain homeostasis, is present in most animals [though not insects or protozoa (5)]. It is composed of cannabinoid receptors and endocannabinoids (endogenous cannabinoids or endogenous ligands), plus synthesis and degradation enzymes. The human body produces chemicals with effects similar to phytocannabinoids in the *C. sativa* plant. These are endocannabinoids, with the 2 main ones being N-Arachidonoiletanolamine (AEA), better known as anandamide, and 2-Arachidonoylglycerol (2-AG). They act as neurotransmitters and interact with specific receptors, namely cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2), which are present in various tissues (central and peripheral). In addition, N-palmitoylethanolamine (PEA), virodamine, and N-araquidonoil-dopamine were recently discovered (7).

A remarkable aspect of ECS activity is its internal regulatory mechanism. Cannabinoid receptors, endocannabinoids and synthesis and degradation enzymes provide negative feedback and retrograde neuronal signaling. Endocannabinoids are atypical messengers: they are involved in inhibitory synapses since they mediate transfer of information from postsynaptic to presynaptic terminals in a retrograde manner and are synthesized on demand (*i.e.,* not stored in vesicles) (12).

Maintenance of homeostasis is a main ECS role, along with modulation of the nervous and immune systems. Other processes involving the ECS include pain and inflammatory conditions, modulation of metabolism and neurological function, appetite, thermoregulation, reproduction, and embryonic development (5).

The ECS exerts its action through a complex mechanism that involves on-demand production of endocannabinoids, such as anandamide and 2-arachidonoylglycerol, in response to specific stimuli. These endocannabinoids act as ligands for cannabinoid receptors, CB1 and CB2, present in neurons, immune cells, and other cell types (13). Activation of these receptors triggers a variety of responses, including modulation of neurotransmitter release, suppression of inflammatory cytokine release, and regulation of intracellular signaling pathways (7). Additionally, the ECS is also regulated by metabolic enzymes, such as diacylglycerol lipase and fatty acid amide hydrolase, responsible for endocannabinoid synthesis and degradation, respectively. These intricate signaling and regulatory mechanisms enable the ECS to modulate a wide range of physiological processes and have crucial roles in homeostasis, modulation of nervous and immune systems, pain responses, inflammation, and other biological processes (5,12).

Discovery of a myriad of long-chain fatty acid derivatives, including primary fatty-acid amides and various N-acylated amino acids and neurotransmitters, sharing molecular targets and inactivating enzymes with endocannabinoids culminated in the concept of the endocannabinoidome. This comprehensive system includes > 100 lipid

mediators, > 20 biosynthetic or inactivating enzymes, and > 20 molecular targets, including previously identified nuclear receptors, ligand-activated ion channels, and orphan G-protein-coupled receptors. The complexity and size of the endocannabinoidome reflects its critical importance in maintaining physiological homeostasis (5,7,12).

Endocannabinoid congeners that accompany AEA and 2-AG in tissues are not inactive, but mainly modulate activity of non-cannabinoid receptors. Receptors such as transient receptor potential (TRP), channel of vanilloid Type-1 (TRPV1), melastatin Type-8 (TRPM8), and G-protein-coupled receptor 55 (GPR55) are modulated by AEA and 2-AG; whereas most others, including peroxisome proliferator-activated receptor-α (PPARα) and G-protein-coupled receptors Types 18, 110, 119, and 132, are not recognized by physiological concentrations of the 2 endocannabinoids. Furthermore, NAEs and 2-MAGs share the same biosynthetic and inactivating enzymes with AEA and 2-AG, respectively (7,12).

Phytocannabinoids are analogous to endocannabinoids by also acting on CB1 and CB2 receptors. New knowledge about the ECS is stimulating interest in phytocannabinoids, as there is evidence of their analgesic, neuroprotective, anticonvulsant, antiemetic, antispasmodic, and anti-inflammatory effects (4,7).

Medical evidence on the benefits of phytocannabinoids is much more limited for veterinary compared to human medicine. Limited evidence supports the therapeutic potential of phytocannabinoids, particularly CBD (4). More information is needed on routes, pharmacokinetics, and pharmacodynamics in various animal species to optimize the safe, therapeutic use of phytocannabinoids.

PHARMACOKINETICS AND SAFETY OF PHYTOCANNABINOID COMPOUNDS IN ANIMALS

Dogs and cats

Interest in phytocannabinoids in veterinary medicine is an impetus to understand their pharmacological action in animals. Phytocannabinoid compounds are apparently safe and well-tolerated, despite interspecies differences in metabolism (14).

Cannabidiol is the most commonly used molecule in pharmacokinetic studies in animals, especially for osteoarthritis and canine idiopathic epilepsy (15). In a landmark pharmacokinetic study, dogs were given 45 or 90 mg (2 doses) or 180 mg orally. With 45 to 90 mg, the increase in the area under the curve (AUC) was proportional to the dose, implying that the pharmacokinetic profile of CBD in this dose range was dose-independent. However, CBD was undetectable in the plasma after oral administration in 3 of 6 dogs, whereas oral bioavailability varied from 13 to 19% in the remaining 3 dogs. Absorption of CBD after oral administration in dogs was considered low and attributed to a first-pass effect (16).

Dogs were given 75 or 150 mg CBD every 12 h (~10 or 20 mg/kg, respectively) for 6 wk, as oral microencapsulated oil spheres, oral CBD-infused oil, or CBD-infused transdermal cream. The CBD-infused oil formulation had the highest maximum concentrations and systemic exposure (AUC) and the least interindividual variation in CBD plasma concentrations. The CBD-infused transdermal cream had the lowest plasma CBD concentrations (17).

In the second study, healthy dogs were given 2.0 or 8.0 mg/kg CBD orally; the 2 doses had similar half-lives (4.2 h) and no psychoactive side effects. Dogs with osteoarthritis were given 2.0 mg/kg, q12h, orally. Combined with anti-inflammatory drugs and some nutraceutical supplements (*e.g.,* chondroitin/glucosamine sulfate), there were no concomitant side effects. However, 56% of dogs had increased alkaline phosphatase, suggesting surveillance (18).

Low, medium, or high doses (2, 5, or 10 mg CBD; and 0.1, 0.25, or 0.5 mg THC per kg of body weight) of a 1:20 THC:CBD herbal cannabis extract were given orally in dogs. Absorption of CBD and THC was rapid, with a mean Tmax of ~2 h for CBD and THC in all dose groups, but the elimination phase was prolonged (> 24 h). Plasma CBD and THC concentrations increased in a dose-dependent manner. Neurological signs, (*e.g.,* hyperesthesia and proprioceptive deficits) were observed 1 to 2 h after administration of high doses, with other adverse events (ptyalism, urinary incontinence, and vomiting) also observed in this group. However, these signs resolved 4 to 6 h after administration. Vomiting (1/6) and coughing (1/6) were observed in the mid-dose group, whereas no dogs receiving the low dose had adverse signs. There was increased production of the metabolite 6-OH-CBD (compared to 7-OH-CBD in humans) (19).

Safety and pharmacokinetics of CBD were studied in 20 healthy beagles allocated into 5 groups: CBD extract (1, 2, 4, or 12 mg/kg per day) or placebo, administered orally, q24h for 28 d. No clinically significant changes in safety outcomes were observed, although 12 mg/kg per day resulted in more adverse gastrointestinal events and higher serum alkaline phosphatase activity. Total systemic exposure to CBD increased in a dose-dependent manner

after acute and chronic administration. The 24-hour plasma concentrations of CBD were also dose-dependent, with a steady state reached after 2 wk of administration (6).

Healthy beagles were given a drug approved for human use (Sativex; GW Pharmaceuticals, Cambridge, UK) with a 1:1 ratio of CBD and THC. Single or multiple sublingual doses were well-tolerated, producing an expected pharmacokinetic profile with maximum phytocannabinoid concentrations within 1 to 2 h after treatment and no adverse effects (20).

Pharmacokinetic and safety studies of these products in cats are very scarce. Phased increases in doses of products containing CBD-rich oil, a THC-rich oil, and an oil in the same 1.5:1 CBD/THC ratios were well-tolerated by cats, with no significant changes in CBC or serum liver enzymes (ALP and ALT). Side effects such as lethargy, hypothermia, ataxia, vocalization, and protrusion of the nictating membrane were more marked with THC (CBD/THC oil and THC oil) compared to CBD oil. Oral CBD appeared to be safe in cats (21).

Another study in cats (n = 8) evaluated physiological and pharmacokinetic responses to a palatable oral paste rich in CBD and CBDA, given q12h for 1 wk. Acidic forms of cannabinoids (*e.g.,* CBDA) were better absorbed than the nonacidic forms such as CBD and THC. In addition, absorption of this specific pasty product may be superior to that of oil bases. No adverse events related to neurological function or behavioral changes were observed and there were no clinically significant serum biochemical changes, suggesting short-term treatment was safe (8).

Several phytocannabinoid products are considered safe for short-term use. Despite few reported side effects, high doses of CBD can elevate alkaline phosphatase in dogs and cats. Sedation, ataxia, and gastrointestinal disturbances (vomiting and diarrhea) were the most common side effects, and in most cases were transient (18).

Cannabis poisoning (with high THC content; *e.g.,* marijuana) is more common in dogs *versus* cats (96 *versus* 3%, respectively). Compared to other species, dogs have a higher density of CB1 receptors in the rhombencephalon (the region composed of the brainstem structures pons and medulla oblongata and the cerebellum), which makes them more susceptible (22). The minimum lethal dose of THC in dogs is > 3.0 g/kg, a value considered high (4). However, the median lethal dose of THC has not been established in these 2 species. Whereas dogs seem to be more susceptible to developing THC side effects, 40 dogs and 69 female rats were given cannabis extracts (1.08 THC: 1 CBD) for 52 wk. At the maximum dose (27 mg/kg THC and 25 mg/kg CBD), prolonged exposure to cannabis extracts caused spontaneous and generalized convulsions that were subclinical to epileptiform discharges in rats, but not in dogs (23).

Birds

A pharmacokinetic study using a single oral dose of CBD (60 or 120 mg/kg) in Hispaniola parrots *(Amazonas ventralis)* revealed highly variable plasma concentrations with a short half-life. Despite a small sample size (4 birds), variability in plasma concentrations was attributed to individual pharmacokinetics, with differences in first-pass metabolism and retention time of food offered (24).

Amazon parrots *(Amazona amazonica)* were given 30/32.5 mg/kg CBD/CBDA using a twice-daily oral dose, respectively, for 7 d. The birds had a consistently quantifiable presence of the 11-OH-THC metabolite, considered the psychoactive metabolite of THC, but not of the other 4 metabolites measured — a main difference between the orange-winged Amazon parrots and mammals studied. Hemp extract was well-tolerated by orange-winged Amazon parrots without significant adverse effects. Target plasma concentrations of 50 ng/mL were achieved for at least 6 h, comparable to other species, but pharmacodynamic studies to determine associations of plasma concentrations and effect on inflammatory mediators are required (25). High doses (compared to those in other animals) had no substantial effects (24,25). Use of other phytocannabinoids in birds, as well as more samples, treatment frequency, and diet type, should be studied.

Horses

Thoroughbreds (n = 12) were given a single oral dose of CBD (0.5, 1, or 2 mg/kg) in sesame oil. Absorption was rapid (30 min) and terminal half-life was ~10 h, longer than in humans (1 to 2 h) or dogs (3 to 4 h). Primary metabolites were 7-COOH CBD and 7-OH CBD in blood and urine, respectively, without adverse effects. Horses appeared to tolerate CBD with no effects on behavior, gastrointestinal tract, or cardiac end points (26).

Horses given CBD in pellets (0.35 or 2.0 mg/kg, orally, q24h for 7 d) had maximum concentrations of 6.6 and 51 ng/mL, respectively. The CBD was well-tolerated, suggesting the need for future studies with higher doses. The product is hemp-based and contained maximum allowable THC (0.3%), which was detected in plasma after a dose of 2.0 mg/kg, which could be of concern in competition horses (27).

In 12 healthy mares given CBD, no side effects were observed, though 8 had increased liver enzymes (9). Furthermore, all mares had decreased plasma calcium concentrations, although ionized calcium remained within normal limits. All values subsequently returned to normal. Plasma CBD concentrations peaked 4 to 5 h after administration (along with feed) and the recommended dosing interval was 12 h. Cannabidiol was detected for ≥ 24 h (up to 96 h) after last administration, and plasma CBD concentrations in horses were lower compared to in dogs and humans after similar dosing (1.0 to 3.0 mg/kg). Horses metabolized and tolerated CBD well and further studies should focus on therapeutic potential (9).

Cannabidiol concentrations were low 30 min after administration in horses given a single dose of CBD (2.0 mg/kg, orally; or 0.1 mg/kg in DMSO given IV). Oral bioavailability of CBD was ~7.92%, lower than in dogs (13 to 19%), likely due to extensive first-pass metabolism in horses. However, there were no adverse effects or analytical and physiological changes (according to CBC or serum biochemistry) (28).

Horses were given 3 increasing doses of CBD paste (0.2, 1.0, and 2.0 mg/kg) orally, at intervals > 1 wk apart. Thereafter, 6 horses were given CBD paste (3.0 mg/kg, orally, q12h for 15 d). The terminal elimination phase began ~132 h after the last treatment, implying a high distribution volume of CBD. Apparently, hepatic metabolism resulted in 7-COOH-CBD. The ratio between the AUC of CBD and 7-OH-CBD was lower, possibly due to metabolism of 7-OH-CBD to 7-COOH-CBD. Concentrations of 7-OH-CBD were higher in urine, with no adverse effects at a maximum dose of 3.0 mg/kg (29).

Cattle

In cattle given 5.0 mg/kg CBDA, the half-life was 14.1 h, with no side effects. However, evaluations of rumen microbiota and metabolism and degradation of phytocannabinoids are necessary (30). *In vitro* conversion of CBD to THC in simulated gastric fluid was reported (31). Rumen microbiota may affect conversion of acid precursors *via* biohydrogenation (32).

In calves given 5.0 mg/kg CBD, q24h, orally, the half-life was 23 h, higher than in the previous study in cattle that used CBDA, and well above the half-life reported in dogs (4.2 h). The maximum concentration of CBD was 0.05 μg/mL at 7.5 h after oral administration. As the forestomachs of young calves are not active, drug absorption may be similar to that in monogastrics. Despite low bioavailability, CBD may have beneficial therapeutic effects in cattle, similar to those in dogs. Although CBD is not approved for use in cattle, plasma concentrations have potential for pain relief, appetite stimulation, and inflammation modulation. Determining pharmacokinetic parameters of CBD could guide future research into its therapeutic efficacy in cattle, as well as appropriate withdrawal periods in livestock and products for human consumption (33).

METABOLISM OF PHYTOCANNABINOIDS

Phytocannabinoids are hydroxylated and glucuronidated by the cytochrome P450 family, primarily in the liver, but also in extrahepatic tissues including the small intestine and brain, with metabolites altered by route of administration (34). Cannabidiol can inhibit several cytochrome P450 enzymes, potentially affecting metabolism of other drugs. The association of phytocannabinoids with some pharmacological classes can promote decreased metabolism, increasing biological activity; and potentiation of some side effects (*e.g.,* sedation). The main classes known to interact with phytocannabinoids, potentiating their effects, are opioid analgesics, antihypertensives (including beta-blockers and calcium-channel blockers), antidepressants (selective serotonin reuptake inhibitors and tricyclic antidepressants), anxiolytics, and steroidal anti-inflammatory drugs (12). Pending better understanding of pharmacokinetics and drug interactions, phytocannabinoids should be used with caution and therapy should begin with low doses (18).

In an *in vitro* study, CBD was metabolized in the dog liver by cytochrome P450 enzymes, mainly CYP1A and CYP2C isoforms. Hepatic metabolism by CYP1A2 was confirmed by a 3-fold increase in intrinsic clearance of CBD in the livers of dogs treated with β-naphthoflavone. The increase was also observed with phenobarbital, but to a lesser extent. Although phenobarbital does not induce CYP1A1 or CYP1A2, it does induce other isoforms, including CYP2C21, CYP3A12, and CYP2B11. However, rifampicin, which selectively induces CYP3A12, showed no induction. Therefore, CYP1A2 and CYP2C21 have an important role in CBD metabolism in the dog liver. Metabolism of CBD in humans is mainly mediated by 7-hydroxylation by CYP2C19 and CYP2C9. In dogs, the main enzyme of the CYP2C subfamily, CYP2C21, is the second-most active enzyme in CBD metabolism. Canine CYP1A2 is important for CBD metabolism in dogs, whereas human CYP1A2 is a minor enzyme in CBD metabolism. In humans, 7-carboxy-CBD is the major plasma metabolite; but in dogs, the AUC values for 7-carboxy-CBD are only ~25% of those for CBD.

Metabolism of CBD by other pathways may be more important for drug clearance in dogs than in humans. Formation of 6-hydroxy-CBD may be important in dogs. Some dogs lack CYP1A2 (genetic polymorphism), potentially increasing CBD bioavailability and effects (34).

THERAPEUTIC INDICATIONS FOR CANNABINOIDS IN VETERINARY MEDICINE

Cannabinoids have been used in veterinary medicine for inflammation and pain as well as dermatology and oncology. Cannabidiol is one of the most studied cannabinoids in animals and has many pharmacological effects, including antipsychotic, anxiolytic, sedative, antiepileptic, anti-inflammatory, analgesic, antiemetic, antidiabetic, and anti-ischemic effects (1,3–7).

Pain and inflammation

Cannabis has been used empirically as an analgesic in all major civilizations of Asia, Europe, America, and the Middle East for > 2000 y (5). Mechanisms of analgesic action of cannabinoids include inhibition of neurotransmitter and neuropeptide release from presynaptic nerve endings, modulation of postsynaptic excitability of neurons, activation of descending inhibitory pain pathways, and reduction of neural inflammation (9). Endocannabinoids and ECS enzymes interact with various receptors, including CB1, CB2, TRPV1, GPR55, GPR119, and PPARα, to inhibit depolarization of primary afferent fibers and modulate mast-cell degranulation (35). In addition to activation of CB1 and CB2 cannabinoid receptors, cannabinoid analgesia may be mediated by other neurotransmitter systems, including noradrenaline, serotonin, peptide systems (orexins, endorphins), and purinergic systems (adenosine) (36).

There are nociceptive modulation systems regulated by cannabinoid agonist-receptor action, and their most important relationship is that linked to the opioid system. Cannabinoid and opioid neuromodulation systems have common features; *e.g.,* opioid receptor antagonists such as naloxone also antagonize the antinociceptive action of cannabinoids (35). The CB1 receptors, together with other receptor systems such as TRP channels, can reduce pain and inflammation by suppressing peripheral sensitization. However, CB2 receptors have a distinct role in modulation of pain and inflammation. Barely present in healthy tissues, CB2 receptors are highly inducible, and their expression is increased after tissue injury or inflammation, similar to the proliferation of opioid receptors in traumatized tissue (35,36).

Endocannabinoids have many roles in pain mechanisms. They are released after tissue injury, inflammation, or disproportionate pain signaling. The purpose of endocannabinoids is to decrease sensitization and pain and suppress the inflammatory cascade. Anandamide has a mechanism of action similar to that of gabapentin, inhibiting calcium channels, which are normally altered in chronic pain (35).

There is much impetus to identify alternative analgesics, *e.g.,* for degenerative joint disease. Standard NSAIDs may not promote adequate pain relief from osteoarthritis and adverse effects may preclude their use, especially in geriatric patients with certain comorbidities, such as chronic kidney disease or gastrointestinal disorders including inappetence, abdominal pain, gastric reflux, inflammatory bowel disease, or irritable bowel syndrome (4,5).

Most studies in dogs with osteoarthritis given CBD demonstrated reduced pain, increased mobility, and improved quality of life (18). In animal models, CBD given before or after the onset of inflammation or arthritis can stop neuropathic pain symptoms and attenuate edema. Five scientific studies regarding treatment of canine osteoarthritis pain reported CBD significantly reduced pain and increased activity, improving quality of life (18,37–40). One study highlighted the significant reduction in pain scale using 20 or 50 mg/d of liposomal CBD (38), and another reported no significant difference between CBD and a control (39). However, CBD pharmacodynamics have been difficult to elucidate. Early reports indicated CBD competed weakly with cannabinoid ligands at the orthosteric site of cannabinoid receptors, implying CBD effects are independent of cannabinoid receptors. However, CBD interacts directly with various receptors, enzymes, and ion channels and directly and indirectly with the ECS (36).

Cannabinoid CB2 receptors modulate inflammation by regulating several anti-inflammatory pathways, including inhibition of pro-inflammatory T-lymphocyte activity (5). Immunomodulatory and anti-inflammatory effects of CBD were demonstrated *ex vivo* in dogs, reducing interleukin-6, tumor necrosis factor-α, and cyclooxygenase-2 (COX-2) expression (40), providing an impetus for further studies.

Seizures and epilepsy

The strongest clinical evidence for the efficacy of CBD as an adjunct therapeutic agent is its treatment of rare childhood epilepsies refractory to treatment; *e.g.,* Lenox-Gastaut syndrome and Dravet syndrome (3).

Intense ECS activity modulates neuronal tone and excitability. Epileptic activity may be associated with changes in

levels and distribution of CB1 receptors in the hippocampus (41,42). The ECS has decreased neuronal excitability and release of neurotransmitters that modulate potassium channel opening and calcium channel blockade (27). Endocannabinoids are released under stimulation and their downregulation has been described in various pathological conditions in humans, including Parkinson's, Alzheimer's, obesity, ischemic brain damage, Huntington's disease, and epileptic seizures. Recurrent seizures can adversely reorganize the ECS and decrease neuroprotective effects (43).

In a randomized, double-blind, placebo-controlled study to evaluate the efficacy of CBD for complementary treatment of canine epilepsy, 2.5 mg/kg of CBD-dominant hemp oil was given orally, q12h for 12 wk (11). The decrease in seizure frequencies was 33% and statistically significant but did not meet the *a priori* threshold of a 50% decrease in frequencies. There was a positive correlation between reduced seizures and higher plasma CBD concentration (11).

Fourteen dogs with refractory epileptic seizures were evaluated in a 24-week randomized crossover study, receiving either 2 mg/kg of CBD-rich hemp extract/CBDA or placebo, orally, q12h. Frequency of epileptic seizures was significantly reduced with CBD-rich hemp extract/CBDA treatment compared to placebo. In addition, the number of dogs with a 50% reduction in epileptic activity during treatment was significantly higher than with placebo. Adverse events were minimal and not significantly different from placebo. The CBD/CBDA-rich hemp in combination with other anticonvulsant drugs appeared to be safe in refractory epilepsy (10).

In a rat model of status epilepticus, CBD combined with appropriate anticonvulsant medications (phenytoin and phenobarbital) reduced the severity and prevalence of generalized seizures. Conversely, CBD alone reduced seizure severity, but did not prevent generalized seizure expression (44).

In a prospective, randomized study to evaluate drug-drug interaction of CBD and phenobarbital in healthy dogs, there were pharmacokinetic variations of CBD but no significant differences, and pharmacokinetic variables of phenobarbital were unaffected, indicating no significant pharmacokinetic interactions (45). During chronic CBD administration, mild gastrointestinal signs were observed in 5 dogs and hypoxia was observed in 5 dogs given 10 to 20 mg/kg per day. All dogs in the study (n = 9) had significant increases in alkaline phosphatase activity over 14 d, with some exceeding the reference range, likely due to the hepatic metabolism of CBD. However, no functional changes in the liver were observed, though monitoring liver function was recommended for chronic CBD use (45).

Anxiety

There are only 3 published studies on the effects of CBD for canine anxiety. The anxiolytic effects of 1.4 mg/kg per day of CBD were compared to those of trazodone in dogs exposed to fireworks noise. The CBD had no anxiolytic effect and did not reduce basal cortisol concentrations in noise-exposed dogs. However, based on its half-life, it may have been administered too early (4 to 6 h) before exposure (46). Twelve dogs from a shelter treated with a CBD-rich oil for 45 d had reduced aggression, albeit at levels not significantly different from those with a placebo (47). Outcomes in both studies may have been limited to the chemical profile of the product, plus low doses. The ability of cannabis to increase or decrease anxiety and emotional reactivity is highly influenced by cannabinoid type and terpenes, as well as dose, time of administration (before or after the event), and setting (neutral or stimulating) (47,48).

In a study on the effects of a single dose of CBD on stress in dogs during separation and car travel, the dose used (4 mg/kg, 2 h before the test) was higher than in previous studies. Significant changes in several stress-related measures (serum cortisol, mean ear temperature, heart rate, heart rate variability, whining, and a stress/anxiety behavioral factor) were observed from baseline to test, with the car ride eliciting a more pronounced stress response. Alleviating effects of CBD treatment varied by measure and test, with canine stress significantly reduced compared to the placebo group in some cases. However, as those dogs did not have clinical anxiety, more research is needed to understand the effects of CBD on canine well-being (48).

Oncologic disease

Cannabis therapy has 2 objectives: direct effects on antitumor activity and improved quality of life (palliative). Antitumor activity of phytocannabinoids has been evaluated in > 100 studies, mostly *in vitro* and preclinical. In animal cell culture studies in nude mice, rats, and rabbits, cannabinoids seem to exert several antitumor properties; *e.g.,* antiproliferative effects, induction of apoptosis and autophagy, antimetastatic effects, immunomodulatory and synergistic effects with conventional therapies (3,5,49). As a standard chemotherapeutic agent, CBD reduced cell viability and induced apoptosis in multiple canine tumor cell cultures, but *in vivo* effects are unknown (49).

Dermatological disease

Skin allergy represents a promising target for cannabinoid treatment in veterinary medicine. Receptors, mediators, and regulatory molecules are expressed by most cellular elements of the skin and a multitude of intricate mechanisms are increasingly recognized to explain the role of the endocannabinoidome in skin homeostasis (50). Cannabinoid CB1 and CB2 receptors had higher immunoreactivity in the skin of dogs with atopic dermatitis (AD) *versus* healthy dogs (13). Cats with dermal hypersensitivity also had higher expression of cannabinoid CB1 and CB2 receptors (50). Cannabis appeared to have therapeutic potential in AD, generalized dermatitis, pruritus, acne, and pain. Cannabidiol has photoprotective, antioxidant, and anti-inflammatory effects on skin. Animal models and pilot clinical trials supported the use of CBD in inflammatory skin conditions, particularly seborrheic disorders (3).

The presence of cannabinoid receptors Types 1 (CB1R) and 2 (CB2R) and various other receptors in healthy keratinocytes supported the hypothesis that the canine ECS has a role in maintaining skin homeostasis by regulation of epithelial cell renewal and differentiation and barrier permeability. There was general regulation of target receptors in the skin of dogs with AD, with significant concentrations of CB2R, TRPA1, and 5-HT1aR, implying ECS is a potential therapeutic target. Perhaps endocannabinoids and cannabis-derived molecules have roles in counteracting skin barrier dysregulation in AD (51). In another study, 32 dogs with AD were given 2.0 mg/kg of an oil with equal proportions of CBD and CBDA, or a placebo, for 4 wk. Despite no significant difference between groups in terms of lesions caused by AD or serum concentrations of various cytokines, scores for pruritus were significantly lower in the treatment group. Therefore, CBD/CBDA may be an adjuvant therapy to reduce pruritus in dogs with AD (52).

Application of an endocannabinoid-like mediator, PEA, significantly reduced antigen-induced papule area in canine models of skin allergy (53). Mast cells and keratinocytes, which are over-activated during inflammatory or allergic conditions, appear to be the target of choice for PEA, modulating and maintaining cutaneous inflammatory responses within physiological thresholds (50).

Nutrition

Hemp and its by-products are relatively inexpensive and increasingly available. Several factors influence research on hemp seeds in livestock diets (7). Plant by-products (stalks, leaves, seeds, seed cake, and flowers after oil extraction) contain protein and fiber (digestible and nondigestible), vitamins, minerals, and essential amino acids, improving feed conversion, weight gain, egg fatty acids (54), and milk production (55).

Feeding whole hemp plants results in high levels of rumen detergent fiber, which is detrimental to rumen health. Most formulations of hulled hempseed or hempseed cake have modest levels of neutral detergent fiber, providing sufficient fiber for rumen fermentation. Hempseed cake is preferred due to its lower fat content compared to hempseed, as fat can also impair rumen function (7).

In 2011, the European Food Safety Authority issued a scientific opinion on the safety of hemp *(C. sativa)* in animal feed. If hemp products are available, maximum inclusion rates are as follows: poultry, 3%; laying birds, 5 to 7% hempseed/hempseed cake; pigs, 2 to 5% hempseed/hempseed cake; ruminants, 5% hempseed cake in the daily ration; and fish, 5% hempseed. The whole plant (or parts of it; *e.g.,* leaves) can be consumed as part of the ruminant diet. It is likely that daily amounts of 0.5 to 1.5 kg of dry matter can be incorporated into the daily rations of dairy cows (5).

When considering inclusion of hemp in cattle feed, it is important to understand its pharmacokinetics and potential biological effects. This information is essential if hemp by-products are to be accepted by the United States Food and Drug Administration and the Association of American Feed Control Officials (30).

In Canada, the Canadian Food Inspection Agency regulates livestock feeds. Currently, hemp and hemp products are not approved as animal feed or feed ingredients. In the future, each hemp product intended for use as an individual ingredient in animal feed (*e.g.,* hemp meal, hemp oil, hemp seeds, hemp silage) will require separate approval. The hemp industry has worked closely with the Canadian Food Inspection Agency to conduct and provide the necessary research studies, trials, and documentation to support approval of hemp products as animal feed (2).

Knowledge of the plant's safety and value for nutritional applications has much scientific and commercial relevance. For hemp to be accepted as an ingredient in animal feed, detailed pharmacokinetics and pharmacodynamics must be known in each species. Furthermore, feeding tests examining lean muscle mass, meat quality, and losses in transport or feeding are needed to ensure that animal-derived food products for human consumption do not contain excessive phytocannabinoid concentrations.

CONCLUSIONS

Although most studies using cannabis in veterinary medicine were recent, the consensus seems to be that its future is promising. Reclassification of the plant and its molecules has become urgent, as have education and training of veterinary practitioners on the ECS. However, knowledge of this therapeutic strategy in animals is scarce, making this research area a fertile ground for investigation of the metabolization, drug interactions, doses, and therapeutic effects of cannabinoids, especially CBD. Legal issues in each country and a lack of control regarding over-the-counter products (online or walk-in stores selling cannabis products) are important obstacles. Veterinarians and animal owners will continue to challenge the pharmaceutical industry to develop high-quality, safe, and effective cannabis-based medicines to provide new therapeutic options and enhance the quality of animal life.

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