Veterinary Cannabis:
Regulatory, Pharmacology, Safety, Applications (Pain & Cancer)

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Introduction

The use of cannabis for animal species is an area of growing interest, largely due to the therapeutic benefits being observed for humans and animals in the era of cannabis legalization. The close relationship humans have with their pets and other veterinary species has led to a renewed interest in the possibility and promise of cannabis to treat health issues similar to those already being treated in humans, in our animal community.

Cannabis sativa L., more popularly known as: Hemp, Marijuana, Mary Jane, Pot, Weed, Ganja, Bhang, Reefer, Dope, or Grass, has been a part of human history since before the written word. Archeological and anthropological evidence supports the fact that cannabis was cultivated by humans since before the beginnings of agriculture more than 10,000 years ago. During the Neolithic period ancient peoples used every part of the plant: The stems and stalks for fiber for cordage and cloth; the seeds which are high in protein and omega 3 fatty acids, for nourishment, and the roots, leaves and flowers for medicinal and ritual applications.

Veterinary medicine has not seen the same advances compared to human medicine for objective, non-biased scientific evidence for the use of medical cannabis in veterinary species. This is due, in part, to the fact that the legalization statutes, state by state, do not provide for similar legal privileges for veterinarians and their patients as physicians have for recommending cannabis for their human patients. However, with the passage of the Farm Bill of 2013, the cultivation and commercialization of hemp (legal low THC cannabis) on a state by state basis began, and with the passage of the Farm Bill of 2018 with the McConnell amendment, the Controlled Substance Act (CSA) Schedule One categorization of the resin derivatives of the hemp plant have been removed, thus opening the door to increased investigation with controlled studies of the benefits and potential risks of veterinary phytocannabinoid therapies.

With the removal of the CSA scheduling of hemp resins, veterinarians will be able to recommend, prescribe, or dispense legally grown low-THC cannabis, which by legal definition is called: “Hemp”. High-THC cannabis, which is called “marihuana” or just “cannabis”, still remains a Schedule One substance, according to the Drug Enforcement agency (DEA); use is only permitted to be recommended by physicians who are certified as medical marihuana physicians. Two states, California and New York have had bills introduced in their state legislatures to give veterinarians the same privileges as human physicians to recommend medical marihuana to their patients. Hopefully this trend will extend to all of the states offering medical marihuana legislation.

Cannabis: The Plant and its Botany

The Latin binomial name for hemp, marihuana or just cannabis in general is *Cannabis sativa* L. It is in the Family Cannabaceae, and shares this family with hops (*Humulus lupulus*) and common hackberry (*Celtis spp*. L). Cannabis is dioecious in that plants can be either male or female, although rarely the plant will contain both male and female reproductive parts. These plants are called hermaphrodites. For resin and seed production, female plants are preferred. When fertilized by the male plant’s pollen, seeds will result, which in seed-oil cultivars is used as a source of omega 3 and 6 oils, and high-quality protein. When the female plants are isolated from the male plant’s fertilization, it results in what has been called: “Sinsemilla” or “without seeds”. The unfertilized female plant directs its reproductive “energies” that would be used to make seeds in the fertilized female plant toward the production of increased amounts of the cannabinoid/terpene resins. For production of medical or recreational cannabis, the unfertilized female plant is preferred. (1)

Strains: In “Cannabis Culture” the use of the designation, “strains”, denotes both the psychotropic effects of cannabis on the human user and certain botanical characteristics of the plant, such as the shape of the

leaves or how the plant grows. Since the "legalization" of medical cannabis in the US and the rise of the industry, breeding practices have interbred strains to create hybrids which blur the lines of distinction between these chemovars.

Strains are subsets of the *Cannabis sativa* L. genome, which contain different distributions of phytocannabinoids, terpenes and flavonoids. The number of possible combinations among these cannabis phytoconstituents is close to infinite. These strains are much like breeds of dogs. All are *Canis familiar*is, but there are definite differences between a Chihuahua and a Saint Bernard, despite the similarity of 99% of their shared genome.

Chemovars: The two primary strains that are described for cannabis are "sativa" and "indica". Each traditionally has had specific characteristics, both physical for the plant and psychotropic impact for the user. A better term than strain would be "chemovar", which describes the specific chemical makeup (fingerprint) of a given cultivar of the cannabis plant.

Sativa is known to be uplifting and cerebral, and in its negative manifestation, speedy and anxiety-causing, with tachycardia and emotional paranoia or nervousness.

Indica is known to be physical, a "body high", but also can be calming, pain relieving and sedating and/or soporific depending on its profile of terpenes and phytocannabinoids.

Terpenes will be described in greater depth later in this paper; they are responsible for the majority of these effects for each strain. To a lesser extent the THC:CBD ratio and presence of other phytocannabinoids will also play a role in the biological impact of cannabis.

Cultivars: This is a general term that is based more on the phenotype of the plant, but also can take into account the chemical constituents of the plant (as is more appropriately defined with the term chemovar). Hemp and "marihuana" are considered cultivars of the cannabis plant, based on the legal definition of hemp which involves a reduced, non-psychotropic amount of THC in the plant (<0.3%). The actual limits of THC in hemp are determined legally by each sovereign nation.

There are three general categories of cultivars of cannabis:

- Plants that produce high amounts of seed oil and protein (food),
- Plants that produce fiber (building materials, biofuels, paper and cloth and cordage)
- Plants that produce large amounts medical resins such as THC and CBD, etc.

***Cannabis sativa* L: The Phytochemical constituents of a Complex Plant**

There are several plant constituents in cannabis of medicinal value. Of most interest are the phytocannabinoids, which consist of more than 100 terpenophilic compounds, found mainly in cannabis, but recently have been described in several other plants in the family Linaceae (Flax), and Asteraceae (Echinaceae [2] and Helichrysum [3]). Other phytoconstituents such as terpenes, terpenoids, and flavonoids also contribute to the medicinal profile of cannabis.

Phytocannabinoids exist in the plant as carboxylic acids and in the acidic form are non-psychotropic. The acidic form is converted to neutral molecular analogs by light, heat and combustion. (1) The phytocannabinoid that has gotten the most attention in this plant is Δ-9 Tetrahydrocannabinol (THC), which provides its psychotropic and some of its medicinal qualities. THC has resulted in cannabis' value, notoriety and illegality. The other phytocannabinoids, which are divided into multiple classes based on chemical structure, are not psychotropic, but contain the majority of the medicinal properties of this plant.

TABLE SUMMARIZING BIO-ACTIVITY of the MAJOR and MINOR CANNABINOIDS (1)

Δ-9-tetrahydrocannabinol (Δ-9 THC)	Analgesic (reduces pain), anti-inflammatory, antioxidant, bronchodilatory, improves symptoms of Alzheimer's disease, benefit duodenal ulcers, muscle relaxant, anti-itch, cholestatic jaundice.

Δ-9 Tetrahydrocannabinolic acid (Δ-9 THCA)	THCA is the acidic or carboxylated form of THC. It is the predominant cannabinoid in psychoactive strains. It is non-psychotropic until activated or decarboxylated, smoked or cooked at temperatures greater than 245°F. Also has medicinal benefits, similar but also separate and different than THC.
Δ-9 Tetrahydrocannabivarin (Δ-9 THCV)	Anti-inflammatory, anti-convulsant, analgesic properties, anti-oxidant, neuroprotective in model of Parkinsons's in one study, improved glucose tolerance and insulin sensitivity in vivo
Δ-8 Tetrahydrocannabinol (Δ-8 THC)	Stable in air, much less psychotropic than Δ-9 THC; At low doses, Δ-8 THC (0.001 mg/kg PO was found to induce appetite stimulation without psychotropic effects.
Δ-8 Tetrahydrocannabinolic acid (Δ-8 THCA)	The carboxylated (acidic) form of Δ-8 THC
Cannabidiol (CBD)	Anti-anxiety, anticonvulsant, Parkinson's disease, Huntington's disease, psychosis, MS, Alzheimers's, cytotoxic for breast cancer, effective against MRSA, reduces oily skin, treatment of addiction
Cannabidiolic acid (CBDA)	Acidic form of CBD, carboxylated form of CBD. Has medicinal properties but not well studied at this point in time.
Cannabichromene (CBC)	Anti-inflammatory, analgesic, antifungal, anti-depressant, Anandamide reuptake inhibitor
Cannabigerol (CBG)	Anti-fungal, GABA uptake inhibitor (calming), antidepressant, analgesic, anti-inflammatory, reduces scales in psoriasis, effective against MRSA
Cannabidivarin (CBDV)	Anti-convulsant
Cannabinol (CBN)	Sedative, effective versus MERSA, helps with burns, reduces scales in psoriasis, helps with breast cancer. May be a degradation product of THC or CBD.

Terpenes/Terpenoids are equally important phytoconstituents of cannabis. These organic compounds are produced by a variety of plants. It is thought they serve a protective function for these plants. They are a significant component in plant essential oils. These molecules are responsible for the aroma of cannabis, and because they, like cannabinoids, are lipophilic, they also cross the blood-brain barrier and contribute to the medicinal benefits of cannabis.

The US FDA considers terpenes and terpenoids to be Generally Recognized as Safe (GRAS), as they are flavor and fragrance components common to human and pet diets. Cannabinoids, terpenes and terpenoids are all produced in the same glandular structure on the cannabis plant, the trichome, from the same chemical precursor, geranyl pyrophosphate. Hops (*Humulus lupus*) is a member of the same Cannabaceae Family as cannabis, and they share many of the same terpenes such as β-myrcene, β-pinene, humulone, and β-caryophyllene. Cannabinoids found in the plant are virtually odorless, emitting only a slight pitch-pine scent.

Flavonoids provide additional anti-inflammatory and anti-oxidant properties to cannabis. There are 21 flavonoids identified in cannabis that are in three different categories: 1) flavones, such as vitexin, apigenin, isovitexin, luteolin and orientin; 2) flavonols such as quercitin and kaempferol; and 3) prenylated aglycone flavanones, which are unique to cannabis (canniflavins A,B & C, which are similar to the prenylnaringenin from hops [*Humulus lupulus*]). These are potent inhibitors of COX2 enzymes, affecting PGE_2 production, thus reducing inflammation through that pathway. (4)

The Biological Impact of Cannabis Phyto-Constituents: The Entourage Effect

The biological effects of cannabis are due to interactions among the three main phytoconstituents: phytocannabinoids, terpenes and flavonoids. This phytochemical interaction has been termed the "Entourage Effect"; it helps to explain the multiple biological activities of the cannabis plant, and the differences that are seen in bioactivity of the different strains of the cannabis plant. The Entourage Effect states that the potency of the whole plant extract is the sum of the interaction of all of the plant constituents involved; this is different than the effect of any individual plant component alone. Another aspect of the Entourage Effect is that there are multiple receptor mediated and non-receptor mediated pathways these phytochemicals can influence in the biomedical activity of the plant as a whole. (5)

The Endocannabinoid System: Ligands, Receptors and Enzymes

Following the determination of the structure of the first cannabinoid, Δ-9 THC in 1964 by Mechoulam, researchers started looking for the membrane receptors that could mediate the activity of the phytocannabinoids and the endogenous ligands to these membrane receptors. In 1988, the first cannabinoid receptor was discovered in the rat brain using a radioactive-labeled THC derivative. This receptor, termed Cannabinoid Receptor 1 (CB1), was determined to be a G-protein coupled receptor with the highest density in the rat cerebral cortex, hippocampus, hypothalamus, cerebellum, basal ganglia, brain stem, spinal cord and amygdala. This receptor is present in all vertebrate species and many invertebrates, indicating that that the endocannabinoid system has been in existence for over 500 million years.

The Endocannabinoid system consists of:

- The endocannabinoid ligand, which binds to the cannabinoid receptor
- The receptor itself
- The enzymes that synthesize and degrade the ligands.

The endocannabinoid system (ECS) has been identified in nearly all animals, from complex mammals like primates, to phylogenetically primitive animals such as members of the Phylum Cnidaria (which used to be called the coelenterates and includes jellyfish). The near universal presence and early emergence of the ECS, evolutionarily, is a strong indicator of its biological importance. Cannabinoid receptors are expressed in most animals, including both vertebrates (mammals, birds, reptiles, and fish) and invertebrates (sea urchins, leeches, mussels, nematodes and others). The most primitive animal an ECS has been observed in is the hydra (*H. vulgaris*), a Cnidarian in the Class Hydrozoa, which is the first animal to develop a neural network. De Petrocellis et al. determined the major function of the ECS in the hydra is to control the feeding response (6). Insects do not have an ECS, and it is thought that is due to the fact that the precursor for the synthesis of endocannabinoids is from arachidonic acid which is lacking in the insect. (7) It is evident from this information that all veterinary species contain an ECS. Therefore, an understanding of the ECS in these species is critical to the development of clinical applications for endocannabinoids and the phytocannabinoids derived primarily from *Cannabis sativa* L.

<u>LIGANDS</u>

Endocannabinoids

Mechoulam, who discovered THC, also discovered the first endocannabinoid, which he called "anandamide" after the Sanskrit word for bliss. Anandamide binds to the CB1 receptor and creates similar effects as the phytocannabinoids naturally occurring in cannabis. A second endocannabinoid was subsequently discovered, 2-arachidonoyl glycerol (2-AG), which Mechoulam characterized from canine gut tissue. (8) There are several other compounds currently under investigation as additional endocannabinoids.

The endogenous agonists for cannabinoid receptors are long-chain polyunsaturated fatty acids that are derivatives of arachidonic acid, and have varying degrees of selectivity for either one or both of the cannabinoid receptors. Endocannabinoids are unlike other neurotransmitters in that they are lipophilic

versus aqueous in nature. They also are not stored, but are manufactured *ad hoc* from precursors in the cellular membrane.

Endocannabinoids are released as calcium levels increase inside the neuron or when G-coupled protein receptors are activated. Endocannabinoids function as neuroprotectants by virtue of their antioxidant activity and by inhibiting calcium influx and excessive glutamate production. There are both cannabinoid receptor-dependent and cannabinoid receptor-independent actions of endocannabinoids.

Activities that are cannabinoid receptor-dependent include cognition, memory, appetite control, emesis, motor behavior, sensory, anxiety, and autonomic and neuroendocrine processes. Endocannabinoids can induce hypotension and bradycardia, inhibit cell growth, affect energy metabolism and modulate immune responses, as well as being involved in fat accumulation, glucose and lipid metabolism. Endocannabinoids can also exert pro-inflammatory actions such as enhancing the cellular migration of eosinophils, neutrophils and natural killer T cells. (9)

Endocannabinoids use a previously undiscovered form of neuronal communication: “retrograde signaling”, which is the opposite to the normal direction of neurotransmitter release from presynaptic neuron to reception on the postsynaptic neuron. Endocannabinoids released from the postsynaptic neuron actually bind at CB1 receptors on the presynaptic GABA neurons to modulate neuronal activity. This novel discovery of retrograde signaling was termed “depolarization-induced suppression of inhibition”, or “DSI”.

DSI helps to explain a number of previously unexplained aspects of brain activity. When you temporarily dampen inhibition, a form of learning termed “long-term potentiation” occurs, which is a process by which information is stored through the strengthening of synapses. It was also found that CB1 receptors can, in some cases, block presynaptic cells from releasing excitatory neurotransmitters. This is true in the cerebellum where endocannabinoids located on excitatory synapses help to regulate neurons involved with motor and proprioceptive control of movement. (10) This helps to explain, in part, the “static ataxia” uniquely observed in dogs only. The canine species have the highest density of CB1 receptors in the cerebellum of any other species studied to date.

Phyto-Cannabinoids

Cannabinoids that are plant-based, known as phytocannabinoids, can bind or interact with the G-Protein coupled receptors of the ECS. THC is the only phytocannabinoid that directly binds with the cannabinoid receptor as a partial agonist. (5)

Terpenes

Terpenes and terpenoids exert strong biological effects by themselves, and have been found to interact synergistically with phytocannabinoids in the treatment of pain, inflammation, depression, anxiety, addiction, epilepsy, cancer, fungal and bacterial infections (including MRSA).

RECEPTORS

Cannabinoid receptors: CB1 & CB2

The CB1 receptor is found in its highest concentrations on neurons that release gamma amino butyric acid (GABA), the main inhibitory neurotransmitter. It is located near the synapse. The discovery of this endocannabinoid receptor was a watershed moment in neurophysiology in that it led to the discovery of the body’s own endogenous cannabinoid molecules (endocannabinoids).

The endocannabinoid receptors evolved along with the endocannabinoids to constitute a naturally-occurring cellular communication system, which is the endocannabinoid system. It is sheer coincidence that the phytocannabinoids found in the cannabis plant resemble the endocannabinoids enough to activate the cannabinoid receptors or influence the ECS through non-receptor mediated activity.

The cannabinoid receptor CB1 is the most abundant G protein-coupled receptor expressed in the brain, with particularly dense expression in (rank order): the substantia nigra, globus pallidus, hippocampus,

cerebral cortex, putamen, caudate, cerebellum and amygdala. This distribution has been determined for the human brain. Immunohistochemical studies in the dog have localized the CB receptors, especially in the skin. (11)

The cannabinoid receptor CB2 is a second G-protein coupled receptor for endocannabinoids. These receptors have been found to be strongly expressed in cells of the immune system, including the microglia, the peripheral nervous system and the organs. CB2 immunoreactivity was found in the B cell zones of lymphoid follicles in the dog, as well as in structures of the skin including mast cells, and hair follicles. (11) CB2 receptors are up-regulated during the early phases of inflammation in cells of the CNS and peripheral tissues, suggesting a role for cannabinoids in the management of inflammatory conditions of those tissues.

The endocannabinoid system's major homeostatic functions were summarized by DiMarzo as: "Relax, Eat, Sleep, Forget and Protect." (12)

The endocannabinoid system has an effect on embryological development, neural plasticity, neuroprotection, immunity and inflammation, apoptosis and carcinogenesis, pain and emotional memory, hunger, feeding and metabolism. (13)

Non-CB receptors

In addition to the receptor-dependent mechanism of action of the cannabinoids, terpenes and terpenoids, their activity can also be mediated through non-receptor dependent interactions. The endocannabinoids exert multiple pharmacological effects through a number of different mechanisms not restricted to modulation of the endocannabinoid system through receptor-ligand binding. A partial list of these non-receptor dependent actions include: (14)

- Transient receptor potential (TRPV1) channel activation
    - Also activates: TRPV2, 3, 4, TRPA1, TRPM8
- Peroxisome proliferator-activated receptor λ (PPAR λ)
- GPR55, GPR18 = atypical cannabinoid receptors
- GPR3, GPR6, GPR12
- Serotonin receptors:
    - 5-HT1A
        - Abnormal-CBD receptor 5-hydroxytryptamine receptor subtype 1A (CBD is an agonist = anxiolytic properties)
    - 5-HT2A
    - 5-HT3A
- Glycine receptors
- GABA-A
- Opioid receptors
- Adenosine membrane transporter phospholipase A1
- Lipoxygenase (LOX) and cyclooxygenase-2 (COX-2) enzymes
- Calcium modulation
- Inhibition of anandamide inactivation by CBD, CBG and CBC

Terpenes and terpenoids exert strong biological effects by themselves, and have been found to interact synergistically with phytocannabinoids in the treatment of pain, inflammation, depression, anxiety, addiction, epilepsy, cancer, fungal and bacterial infections (including MRSA). (5)

Enzymes to Recycle Endogenous Ligands

Fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) which are enzymes of the endocannabinoid system, deactivate the endocannabinoids AEA and 2AG and their congeners, respectively. Systemic endocannabinoid levels, which are referred to as the "endocannabinoid tone", are tissue dependent and are regulated by these two enzymes.

Its important to note here, that cannabidiol and phytocannabinoids other than THC inhibit these enzymes which prolongs systemic tissue exposure to the endocannabinoids.

CANNABIS AND CANCER?

First a little history:

> A 2500 year old mummy was unearthed in Siberia in 1993. Known as the "Siberian Ice Maiden", her burial chamber contained, among other things, a pouch of cannabis. MRI imaging revealed that the princess had a primary tumor in her right breast with enlarged lymph nodes and metastatic disease. It has been speculated that the cannabis was used to manage her pain and other symptoms, or even had been used as a treatment for her breast cancer. (15)

What if cannabis *did* cure cancer? Cannabis has had the popular "reputation" that it can cause cancer to go into long term remission. and that claim is repeated frequently on the internet. You have on the internet the phenomenon of "Rick Simpson Oil" (RSO), in which Mr. Simpson has cured his own cancer taking a concentrated oil extract he made himself from the cannabis plant. This recipe is available on the internet and there are claims from a number of people who had cancer who tried this approach and reportedly cured themselves.

Since the mid-1970's, researchers have been studying the effects that both endogenous and exogenous cannabinoids have on cancer, *in vitro* and *in vivo*. With the ongoing legalization of medical cannabis, state by state in over 50% of the US, this topic has now become open to increased investigation. The cannabis plant, the source of plant-based cannabinoids, has been illegal for 70+years in the United States and most of the world. The two plant-based cannabinoids, cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC) are the cannabis plant compounds at the center of these research efforts. Another factor that has been an impediment to veterinary research, specifically, is the lack of state medical cannabis legislation that would allow veterinarians to work with medical cannabis with their patients. This legislation would give veterinarians the same privileges that human physicians have with regard to recommending medical cannabis treatments to their patients.

<u>The Endocannabinoid System And Cancer</u>

The endocannabinoid system (ECS) is a recently discovered signaling system made up of: 1) endocannabinoid receptors, which are G-protein coupled receptors distributed widely in the central nervous system and immune systems, as well as other bodily systems; 2) Intrinsic lipid ligands, the endocannabinoids AEA (N-arachidonoylethanolamide or "anandamide") and 2-AG (2-arachidonoylglycerol); and 3) the transporters and biosynthetic and degradative enzymes. The endogenous ligands will also bind to other receptors such as the TPRV1 (vanilloid or capsaicin) receptor, the GPR55 "orphan" receptor, the peroxisome proliferator-activated receptor (PPAR), and the 5-HT_{1A} receptor as well as others.

The endocannabinoid system can produce anti-neoplastic effects in a number of other types of cancers, such as cancer of the breast, prostate, bone, skin, brain (gliomas), and lung. (16) Studies have found that by removing the activity of the CB1 and CB2 receptors experimentally, a higher incidence of cancer will result. In mouse models of cancer, the genetic ablation of CB1 and CB2 receptors increases ultraviolet light-induced skin carcinogenesis. CB2 receptor (found mainly in the immune system) *over-expression* enhances predisposition to viral leukemia. CB2 receptors can be found on tumor cells.(17)

The pharmacological activation of cannabinoid receptors can reduce tumor growth. Upregulated endocannabinoid-degrading enzymes have been observed in aggressive human tumors and cancer cell lines, indicating that the presence and signaling action of endocannabinoids, and perhaps phytocannabinoids, can have a tumor suppressive role.

When CB1 receptors have been deleted in a genetic mouse model of colon cancer, it was found that tumor growth was increased. Precancerous lesions in the mouse colon, induced by the chemical azoxymethane, could be reduced with increases in endocannabinoid levels. With reduced expression of the

endocannabinoid-degrading enzyme, monoacylglycerol (MAGL), prolonging elevated serum levels of endocannabinoids, tumor growth was inhibited in xenografted mice. The precise signaling mechanisms that regulate cannabinoid-induced cell death or cell proliferation continue to be under investigation. We are still discovering the many details that will help to clarify the role the endocannabinoid system plays in tumorigenesis and tumor suppression.

Phytocannabinoids And Cancer

The anti-proliferative properties of cannabis were first reported 40 years ago when it was shown that THC inhibited lung adenocarcinoma growth *in vitro* and *in vivo* in mice. No other research resulted from this study for well over 20 years, due to the prohibition around the cannabis plant. In the last 20 years, though, we have seen an emerging body of investigation, mainly using *in vitro* models of different cancers, to further elucidate the mechanisms whereby the ECS has an impact on cancer cell proliferation, angiogenesis and metastasis.

Since the late 1990s, following the discovery of the ECS, these studies have shown that the cannabinoids have an anti-tumor effect in a wide variety of experimental models of cancer. These studies have found that the pharmacological stimulation of CB receptors is anti-tumorigenic. (18)

A number of cannabinoids (endo-, phyto- and synthetic) have been shown to have this activity, including 1) THC; 2) CBD; 2) 2-AG and anandamide; 3) synthetic cannabinoid receptor agonists with equal affinity for both CB1 and CB2, such as WIN 55, 212-2, and HU-210; 4) synthetic cannabinoid receptor agonists with a higher affinity for CB1 such as methandamide; and 5) synthetic cannabinoid receptor agonists with a higher affinity for CB2, such as JWH-133. The anti-neoplastic mechanisms of action of cannabinoids have been established through examination of the pharmacological impact of cannabinoid receptor agonists on tumor growth. (17)(20)

Randomized clinical trials with cancer patients who have naturally-occurring disease are still lacking from the literature, but there is sufficient evidence from case studies to say that the phytocannabinoids, THC and CBD, have been found to have activity against a number of tumor types: breast, bone, glioma, leukemia/lymphoma, lung, colon, prostate and thyroid. (16)(21)

Induction of Cancer Cell Death and Anti-Proliferative Effects (17)

The cannabinoids have been found to induce apoptosis by means of CB1 and CB2 stimulation of the synthesis of the pro-apoptotic sphingolipid, ceramide. In studies performed in vitro with THC-resistant and THC-sensitive glioma cells, it was found to up-regulate the expression of the stress-regulated protein P8 (AKA: NUPR1). This protein is a transcription regulator and involved in the control of tumorigenesis and tumor progression. In these studies, THC also impacted the endoplasmic reticulum (ER) stress-related transcription factors ATF4, CHOP (AKA: DDIT3) and TRIB3. ER stress response is an attempt by the endoplasmic reticulum to re-establish homeostasis.

The stress response becomes activated in response to Ca^{+} depletion, oxidative injury, a high fat diet, hypoglycemia, viral infections and exposure to certain anti-cancer agents. ER stress reduces the protein load on the endoplasmic reticulum by shutting down protein translation and gene transcription with the goal of increasing the ER protein-folding capability. When this stress response fails to restore homeostasis, cell death can ensue, usually through intrinsic apoptosis, but through a different pathway can result in *autophagy*, which is another cause for cancer cell death.

Autophagy is an attempt of the cell to correct its imbalance, and if successful, the cell will continue to live. So, autophagy is not always an effective path to cancer cell death. Interestingly, it has been found that autophagy is “upstream” to apoptosis in the mechanism of cannabinoid-induced cancer cell death. Blocking autophagy prevents cannabinoid-stimulated apoptosis, but apoptotic blockade prevents apoptotic cell death but not autophagy.

In addition to inducing cancer cell death through autophagy or apoptosis, cannabinoids also have been found to have an anti-proliferative effect by inducing cell cycle arrest. The effect of cannabinoids on hormone-dependent tumors may be due to their interference with activation of growth factor receptors.

Cannabinoids can also down-regulate other cancer cell growth factors such as: PIGF, BFGF, SDF-1, Ang-2, leptin, interferon-γ, and thrombopoietin. Cannabidiol, or CBD, is a cannabinoid that does not bind to CB1 or CB2 receptors directly, yet uses many alternate pathways to influence the endocannabinoid system.

CBD has been observed to promote the apoptotic death of cancer cells, independent of CB1 and CB2 receptors. Its mechanism of action, which has not been completely worked out, promotes the production of reactive oxidative species in cancer cells as well as an increase in the other endocannabinoids through inhibition of FAAH and MAGL.

Inhibition of Angiogenesis, Tissue Invasion and Metastasis (17)

The activation of the vascular endothelial growth factor (VEGF) pathway in cancer cells is known to induce angiogenesis. It has been found that cannabinoids down-regulate the two main VEGF receptor (VEGFR1& VEGFR2) pathways through reduced production of VEGF. VEGFR activation is decreased as a result of the reduced amount of its ligand, VEGF. Activation of the CB receptors in vascular endothelial tissue inhibits proliferation and migration and induces apoptosis, in addition to activating endothelial cells. Thus, cannabinoid activity results in a more normalized tumor vasculature with smaller and/or fewer vessels that are less “leaky”, therefore less likely to result in metastasis.

Phytocannabinoids have been found to reduce metastasis in animal models for glioma, breast, lung and cervical cancers grown in tissue culture. Tissue invasion, which is required for metastasis, is regulated by the extracellular proteases (MMP2) and their inhibitors (TIMP1), which are modulated by cannabinoids.

Clinical Use of Phytocannabinoids in Cancer Patients

Although historical and anecdotal evidence suggests that cannabis can be used to treat clinical neoplastic disease, carefully-controlled clinical trials of cannabis as a cancer treatment are rare to non-existent. Currently in the United States, THC and CBD are both considered to be Schedule One controlled substances. Researchers need a special Schedule One registration with the Drug Enforcement Agency of the United States to conduct research using these cannabis resins, and the only legal source of cannabis for clinical trials is the National Institute for Drug Abuse (NIDA), which makes acquisition of these plant extracts difficult to obtain. These factors all have made it difficult to conduct clinical studies into the effectiveness of cannabis for treating cancer.

Ladin (22) in his review article describes a number of in vitro and in vivo studies using cannabinoids in human patients with glioblastoma. Here they are summarized:

- Phase 1 clinical trial of THC in GBM patients indicated a good safety profile
- Intra-tumor injection of THC in nine patients with actively growing recurrent GBM decreased tumor cell proliferation and induced apoptosis
- Cannabinoids promoted the survival of healthy oligodendrocytes, astrocytes and neurons
- The anti-neoplastic effect of THC was enhanced when combined with CBD
- THC:CBD in combination with standard GBM alkylating chemotherapy drug Temozolomide (TMZ) was more effective in reducing tumor size than any one of these agents alone.
- THC:CBD treatment of GBM tumors in mice enhanced the cytotoxicity of ionizing radiation

GW Pharmaceuticals’ sublingual drug Sativex™ contains a 1:1 ratio of CBD:THC. In a study including 21 adult patients with histopathologically-confirmed GBM, subjects were receiving TMX chemotherapy and also received 27 mg THC and 25 mg CBD daily. The control group only received the TMZ. They had a 44% 1 year survival rate. The cohort of subjects receiving the TMZ and Sativex™ showed an 83% 1 year survival rate with a median survival of over 662 days as compared to 369 days in the control group. (23)

Cannabinoids can express anti-neoplastic activity through binding with the CB1 receptor (This is for THC, anandamide, 2-AG). Phytocannabinoids other than THC (CBD, CBG, CBC, and others) do not bind to the CB1 or CB2 receptors but inhibit the enzymes (FAAH, MAGL) that degrade anandamide and 2-AG, thus causing prolonged binding of endocannabinoids to the cannabinoid receptors, which also has an anti-neoplastic effect.

The brain has the highest density of CB1 receptors in the body. Numerous studies in vitro and in animal models suggest that cannabinoids can inhibit gliomas. (17) It has been found that other cancer cell lines are also inhibited by cannabinoids *in vitro*, such as adenocarcinomas of the lung, breast, colon and pancreas, and also myeloma, lymphoma, and melanoma. Cannabis can enhance the activity of certain chemotherapeutic agents, and through the down-regulation of p-glycoprotein may also be able to reduce chemotherapy resistance.

The majority of studies evaluating cannabis in cancer patients have been to evaluate its ability to address symptoms of cancer or cancer therapies, such as pain, nausea, or anorexia. These studies have found that cannabis can work well for cancer pain. Cannabinoids have been found to work synergistically with concurrent opiate medication for a better pain response in the cancer patient. (24) One of the highest callings for medical cannabis in benefiting the cancer patient is its ability to control nausea, often better than the existing anti-emetic armamentarium. The synthetic cannabinoid dronabinol (Marinol™) was originally designed for this purpose, and can work well. Marinol™ is a 100% synthetic THC analog, and some persons taking it report hallucinations. This side-effect is due to the lack of modulating phytocannabinoids such as CBD or CBG to temper the psychoactivity of the Marinol™.

There is good evidence that plant-based cannabinoids work better to relieve nausea without the potential for adverse events. This is due to the tempering effect that a full spectrum cannabis extract with its full complement of phytocannabinoids has on the psychotropic effect of THC. (25) Appetite stimulation is the third reason that cannabis has value to the cancer patient. Famous for creating “the munchies”, THC and CBD can help to relieve nausea and also increase appetite better than many of the existing aperient formulations.

Cannabis is the only anti-emetic that is also an appetite stimulant, although for anorexia-cachexia one study found it to be no better than placebo. This RCT was placebo-controlled to compare cannabis with the synthetic cannabinoid, dronabinol (Marinol™) in 243 human patients with cancer-related anorexia-cachexia syndrome. It was found that there was no difference among any of the two treatments and the placebo with respect to affecting appetite or quality of life. (26) Anecdotally, veterinary oncologists are observing that THC does not stimulate appetite in more than 50% of dogs, although this may be dose-dependent. Cats have been observed to exhibit a better appetite response to THC than dogs.

Terpenes, which make up 10% of the total production of active molecules by the cannabis plant, are an integral part of the “Entourage Effect” that contributes to the clinical efficacy of cannabis. Certain terpenes, such as limonene has been shown to cause apoptosis of breast cancer cells. Additionally, for cancer pain, terpenes contribute substantially. Both phytocannabinoids and terpenes reduce inflammation and pain via inhibition of COX-2 and PGE2α as well as a number of other mechanisms of action such as the TRPV1 pathway.

<u>Veterinary Cancer Patient Cannabis Considerations</u>

For the veterinary cancer patient, legal constraints to the use of THC must be accounted for in a reasonable and safe fashion. Veterinarians cannot prescribe or dispense THC. However, pet owners will come to a veterinarian asking for help using cannabinoids to treat their pet’s cancer who have full access to state-legal dispensaries and the THC products they sell. The veterinarian should explain the risks and the problems with the current legal landscape, and the potential to send a patient to the ER with high doses of THC. The vet should explain that they cannot legally recommend or prescribe these Schedule One controlled

substances. If the owner persists, then the veterinarian can give advice that will help to create a successful outcome free of unwanted side effects.

Studies in the 1970s discovered that dogs have a very high density of CB1 receptors in their hind brain that govern balance and cardiovascular function. (27)(28) This is why a few dogs who are naïve to THC, when exposed to a sufficiently large amount of THC (especially when in combination with chocolate, as is commonly found in human "edibles"), have had reported deaths. (29)

These early studies found that dogs can develop tolerance to THC's adverse effects in about a week when THC is introduced in small amounts initially and gradually increased over time. Once tolerance is achieved, dogs were able to handle escalated doses 100 times higher than the original dose that had caused the adverse response. Once tolerance has been developed, the canine patient can then tolerate larger doses if their condition warrants dose escalation.

Studies (30) and reports from human and veterinary oncologists, veterinarians, medical cannabis physicians, pet owners, and human cancer survivors, although anecdotal, indicate that the most successful approach to the use of cannabinoids in the cancer patient involves the oral use of a combined formula containing both THC and CBD, usually in a 1:1 ratio. This combination enhances the anticancer activity of the formula as these two cannabinoids have similar but different mechanisms of action against cancer, and work together synergistically. Using a "ratio approach" to the blending of CBD with THC can help to reduce the dose of THC that is needed to inhibit tumor growth. The use of CBD also reduces the unwanted side-effects of THC, such as psychoactivity, convulsions, discoordination, and psychotic effects in humans and dogs, and in the dog, specifically, static ataxia.

The best approach to the blended use of THC and CBD from cannabis in a cancer patient is to use products that have THC and CBD formulated into a specific ratio of CBD:THC. Ratios can help to reduce the side-effects from THC and maximize the anti-neoplastic activity of the treatment. The protocol is to first start with cannabis that is hemp, which would have a ratio of CBD to THC of about 25:1.

The use of this small amount of THC present in the hemp will help to "tolerize" your patient to the larger doses of THC that will be used for anti-neoplastic effects in a 1:1 ratio of CBD:THC. CBD-dominant strains of cannabis with very low THC, but higher than hemp levels of THC (>0.3%) can be used to increase the patient's tolerance to the higher doses of THC that are needed for cancer.

In several oncology cases reported to this author by a board certified veterinary oncologist who helps clients who are giving their pets THC and CBD for cancer to do that safely and reduce harm, found that by starting low, going slow, and staying as low as possible with the THC dosage, that tumor remission was achieved in each case with less than 1.0 mg/kg BID of THC. (31)

Once the dog has been on the hemp for a week at a dose of 0.5-1.0 mg/kg BID of CBD, it should be tolerant of increases in its THC dosage, which will better address the anti-neoplastic effects of cannabis. Starting dosages for the THC are also in the 0.1-0.5 mg/kg BID range, with the lower doses being less likely to cause adverse events. In this author's experience, patients who were taking 100% CBD for their tumors, with zero THC, would show an observed reduction in the size of the mass in about 6 weeks for that reduction to be substantial (as documented with photographs). The dose used in these successful cases was 0.5-1.0 mg/kg BID of CBD. Not all tumors respond to this dosage, but in those that do respond it is remarkable to watch.

SUMMARY

The available literature suggests that the endocannabinoid system can be targeted to suppress the evolution and progression of certain types of cancer and the pain, emesis and appetite syndromes associated with these diagnoses and treatments. Despite the fact that there are no controlled clinical trials of cannabinoids in cancer patients as anti-neoplastic agents, there still is sufficient evidence to support the use of cannabinoids for veterinary patients with cancer in general, although specific tumor types may be more or less resistant to their beneficial effects.

The biggest problem to date is the legal landscape, since cannabinoids have been shown to be quite safe when given in controlled amounts. Large population studies in humans have found no correlation between smoking cannabis and increased risk of respiratory symptoms/chronic obstructive pulmonary disease or lung cancer. (32) The same has been found to be true with bladder cancer prevention and cannabis. (33)

Phytocannabinoids can be used concurrently with chemotherapy and have been found to not interfere with chemotherapy efficacy in the tumors and chemotherapy agents measured. (34) Determining the effective dosage for an individual patient and tumor type will improve outcomes, and is work that still needs to be done. Cannabinoids can induce autophagy, apoptosis, cell cycle arrest, reduce angiogenesis, tissue invasion and metastasis, without affecting normal cells. Cannabinoids can reduce pain, nausea and improve appetite. All of these actions make the use of cannabinoids for cancer very attractive to both the practitioner and the pet owner.

BIOMEDICAL ACTIONS AND POTENTIAL VETERINARY APPLICATIONS (43)

Pain, Inflammation and Immunomodulation

- Effective for both acute and chronic pain by centrally and peripherally modulating nocioception
- CBD affects T-cells resulting in a mild generalized immunosuppressive effect
- CBD has been found to have potential benefit for arthritis and psoriasis in humans

Epilepsy

- CBD attenuates seizures in experimental models of epilepsy in animals
- THCV inhibits CB1 receptor activity resulting some anticonvulsant activity

Anxiolytic

- CBD exerts benzodiazepam-independent activity, postulated to be via post-synaptic 5-HT1A receptors

Neuroprotection

- CBD acts as an antioxidant and as such has been suggested for Alzheimer's, Parkinson's and Huntington's diseases.

Anti-emesis

- CBD in animal models has been found to be effective for the control of vomiting that is unresponsive to 5-HT-3 agonists such as metoclopramide or ondansetron

Diabetes Mellitus

- CBD inhibits development of diabetes in experimental models of diabetes in mice, and causes eduction of pancreatic inflammation in pancreatitis. (Mo et al. 2014).

Bone formation

- Cannabinoids stimulate the stem cells responsible for fracture healing and bone formation, as well as reducing bone loss by controlling bone reabsorption

Cancer

- Many of the cannabinoids have anti-apoptotic effects and reduce neoplastic proliferation in selected tumor cell lines
- Anecdotal reports from both human and veterinary patients indicate the potential for complete remission and possibly even cure of a number of different neoplastic diseases

Anti-microbial

- Both CBC and CBG have potent anti-bacterial effects including against MERSA (MIC of 0.5-2 mcg/ml)

VETERINARY EVIDENCE-BASED APPLICATIONS

SURVEY-STUDIES

1. Pet Owner Experiences with Hemp Products (Kogan1)

This was an on-line survey of pet owners who visited a CBD for pets website.

Results:

Most common uses for hemp products reported by pet owners on the survey:

(D=dog; C=cat)

Listed in descending order of frequency of survey response

- Pain management and arthritis (D)
- Anxiety (D&C)
- Cancer (D)
- Inflammation (C)
- Disturbed sleep (D&C)
-

2. Demographics and Dog Owner Perceptions of Cannabis (Kogan 2)

This second study was a follow-up on-line survey of pet owners using social media (1068 respondents)

Results:

Most common uses for hemp products reported by pet owners on the survey:

(NOTE: Dog owners only were included in this study)

Listed in descending order of frequency of survey response:

- Pain relief
- Anxiety
- Reduction of inflammation
- Epilepsy
- Cancer
- Arthritis
- Allergies

Compilation of Veterinarian Uses of Cannabis in their patients

Although this is not controlled data, this author has personally spoken with or corresponded with hundreds of veterinarians who have been using industrial hemp extracts in their practices over the past 2 years. Over 40,000 bottles have been distributed to veterinarians in this time period. These uses parallel those detailed by the two previous surveys described above.

- Pain management
- Anxiety and behavior problems

- Epilepsy
- Cancer treatment
- Cancer treatment side-effects
- Inflammatory bowel disease

As the clinical use of cannabis becomes more commonplace, we are seeing more detailed studies emerging to help fill in the many blanks that are still lacking in our comprehensive understanding of the safety and efficacy of this very popular and quite effective emerging veterinary plant-based therapeutic. The following studies are some of the few published studies in the dog. An article in the British Medical Journal by Walter Dixon MD an early pharmacologist is the first published evaluation of cannabis in dogs and cats, written just before the turn of the 20th century in 1899. (35) It is the first publication where static ataxia is described. Since then, studies have been few and far between due to the controlled substance status of cannabis, and the negative effect that has on research study funding.

EARLY STUDIES IN THE DOG

Research performed in the 1970's by the Department of Defense explored whether marijuana could be "weaponized". Research animals were administered radioactive-labeled THC intravenously at escalating dosages. As a result, researchers found that dogs, as compared to pigeons, monkeys, guinea pigs, rats and mice, had the highest concentration of THC (now known to be bound to CB1 receptors) in the cerebellum. The canine THC detected was more dense than in any of the other species studied (27)

Dogs, as compared to other species studied developed a pathognomonic neurologic condition termed "Static Ataxia" which results from the THC binding to CB1 receptors in the dog's cerebellum. Previous studies had found that the minimum dose of THC administered IV to create static ataxia was 0.5 mg/kg IV.(28)

Tolerance to the "behavioral" effects of THC in the dog developed after daily injections were given. McMillan found that a dose of 2 mg/kg IV produced marked static ataxia, evidenced by "swaying movements, hypersensitivity to moving objects and prance-like foot placement." Most of the dogs in this study group developed tolerance to these adverse neurological effects rapidly after the first administration of 2 mg/kg of THC. Subsequent injections continued to increase the degree of tolerance to THC. The magnitude of tolerance developed in these canine studies was in excess of 100 fold. (36)

VETERINARY UNIVERSITY STUDIES

COLORADO STATE UNIVERSITY

1*. Pet Owner Experiences with Hemp Products (37)*

This was an on-line survey of pet owners who visited a CBD for pets website.

Results:

Most common uses for hemp products reported by pet owners on the survey:

(D=dog; C=cat)

Listed in descending order of frequency of survey response

- Pain management and arthritis (D)
- Anxiety (D&C)
- Cancer (D)
- Inflammation (C)

- Disturbed sleep (D&C)

2. *Demographics and Dog Owner Perceptions of Cannabis (38)*

This second study was a follow-up on-line survey of pet owners using social media (1068 respondents)

Results:

Most common uses for hemp products reported by pet owners on the survey:

(NOTE: Dog owners only were included in this study)

Listed in descending order of frequency of survey response:

- Pain relief
- Anxiety
- Reduction of inflammation
- Epilepsy
- Cancer
- Arthritis
- Allergies

3. *Pharmacokinetics of cannabidiol administered by 3 delivery methods at 2 different dosages to healthy dogs.*

This study was designed to determine the pharmacokinetics of CBD in healthy dogs. A sample population of 30 healthy research dogs were assigned to receive 1 of 3 different formulation at a dose of 75 or 150 mg q12 h for 6 weeks. The dosage formats were: 1) Liquid oil infusion administered to the oral mucosa; 2) oral capsules with microencapsulated oil beads; 3) transdermal application. Serial CBD plasma concentrations were measured over the first 12 hours and repeated at 2, 4, and 6 weeks. Greater plasma concentrations were measured with the oral CBD-oil infused formulation. The plasma half-life of CBD administered via this route after the 75 mg and 150 mg doses respectively were 199.7 +/- 55.0 and 127.5 +/- 32.2 min. This study found that blood levels are dose proportional, as expected and the oral liquid CBD absorbed transmucosally was the superior formulation of the three formulations tested, with orally administered microencapsulated beads the second-best formulation in terms of pharmacokinetic profile. The CBD had a peak at 2 hours post ingestion and a half life of 4-6 hours. (39).

4. *A report of adverse effects associated with the administration of cannabidiol in healthy dogs.*
A study that is currently *in press* for the Fall of 2018 was performed at Colorado State University's College of Veterinary Medicine, Neurology Department. The principal investigator, Stephanie McGrath, who is a veterinary neurologist and Assistant Professor at CSU's Veterinary Teaching Hospital, conducted a 6-week high dose evaluation of the tolerability of two high doses of CBD in healthy beagle dogs. A sample population of 30 healthy Beagle dogs were randomly assigned to receive one of three formulations: Microencapsulated oil beads, CBD infused oil or CBD infused transdermal cream for 6 weeks. Two dosage tiers were evaluated in this study, 10 mg/kg/day and 20 mg/kg/day. These dosages far exceed the dosages used in the two efficacy studies that followed this by a factor of 2X and 4X greater. The two efficacy studies evaluated the use of CBD for refractory epilepsy and osteoarthritis in the dog.

Complete blood counts, chemistry panels, urinalysis and pre- and post-prandial bile acids were performed at 0, 2, 4 and 6 weeks. Elevations in alkaline phosphatase double the high end of the reference range (140 IU/L) were observed in some dogs (11/30:36%) after being on the CBD for 4 weeks, although it did elevate in some dogs at 2 weeks, especially at the higher dosing tier. Long term liver toxicity was not evaluated in this study, although bile acids and liver enzymes remained normal for all dogs throughout the study. None

of the dogs receiving the transdermal formulation developed elevated alkaline phosphatase values. All dogs experienced mild diarrhea, although there was no correlation with formulation or dose. 6 out of the 30 dogs developed vomiting, but there was no significant difference between the occurrence of vomiting and CBD dose or formulation.

Erythematous pinnae were the next most commonly reported clinical sign in this study. These otic changes were seen in 36% of dogs with the otic changes becoming more severe after 2 weeks in the 10 mg/kg/day dosage group for all three formulations. The transdermal crème had more incidences of otic changes than either the transmucosal or oral routes of administration, which is understandable since the transdermal crème was applied to the inside of the pinna. Less common findings included ocular discharge in 10/30 dogs (33%) and nasal discharge in 10/30 dogs (33%). 5 dogs (17%) had salivary staining on their feet and occasionally ventral abdomen. Two dogs had spontaneous prolapsed glands of the nictitans. One dog had a transient elevated body temperature (104.2^{o}F). It was also observed that some dogs would salivate following administration of the CBD-infused oil formulations at both doses. The study concludes that CBD seems to be well tolerated in the dog at these high dosages, but emphasize that a larger and longer in duration safety study is needed to evaluate the very long-term effect of CBD on the liver and its association with diarrhea (40) (41).

5. *Efficacy Studies: Pilot Trials for Osteoarthritis and Refractory Epilepsy* (*in press*)

Osteoarthritis

The osteoarthritis (OA) wing of this efficacy study consisted of 24 client-owned dogs with clinical evidence of OA radiographically and who had an identifiable lameness. A double- blinded, randomized, placebo-controlled study design was utilized, with each study group receiving medication for 6 weeks and a placebo for 6 weeks. The treatment group received 2.5 mg of CBD oil q 12 hours. Gait analysis and an activity monitor were used to gain objective data, and a behavioral questionnaire was given to the dog owners which provided subjective information. The study results for OA were not yet available at the time of this publication. (40)

Refractory Epilepsy

The epilepsy segment of the study consisted of 16 client-owned dogs who were diagnosed with idiopathic refractory epilepsy, having 2 or more breakthrough seizures per month while receiving conventional anti-convulsant therapies. Inclusion criteria included a normal neurologic exam and a normal epilepsy workup with an MRI and CSF analysis. Nine (9) dogs were randomly assigned to the treatment group and 7 to the control (placebo) group. The treatment group received 2.5 mg/kg CBD oil q 12 hours by mouth. The control group received placebo oil for 12 weeks. Study subjects were required to stay on their standard anticonvulsant drugs (AED). Routine blood work and CBD levels were determined every four weeks. AED levels were measured at the conclusion of the trial.

67% (6/9) of the dogs in the treatment group experienced a greater than 40% reduction in average monthly seizures during the study; whereas only 29% (2/7) of the dogs in the control group had a greater than 40% reduction in average monthly seizures.

Elevations in alkaline phosphatase (ALP) were recorded for the treatment group, and one dog in the control group. The single control dog had previously measured elevations in ALP so this elevation was not considered to be relevant to the study. 6 dogs (67%) in the treatment group had elevations in ALP measured at the end of the study. The mean ALP value was 619 IU/L (range 15-140 IU/L).

AED concentrations in the treatment group for phenobarbital decreased in 2/7 dogs (29%) and increased in 5/7 dogs (71%). In the control group phenobarbital levels decreased in 3/5 dogs (60%) and increased in 2/5 dogs (40%); there was no significant change in either group. This is an interesting finding to note, because there has been a concern that CBD, which is metabolized through the P450 group of cytochromes, might

interfere with the drug disposition of pharmaceuticals that also are metabolized through that pathway. From the results of this pilot study, that effect is not apparent, at least with respect to phenobarbital levels.

Potassium bromide (KBR) levels in the treatment group decreased in 2/3 dogs (67%) and increased in 1/3 dogs (33%). In the control group KBR levels decreased in 1 out of 2 dogs (50%) and increased in 1 out of 2 dogs (50%). There was no significant change in either group, although the total number of study subjects was low in this pilot study. This research and the osteoarthritis section of this study have not yet been published, pending the results of the plasma analysis of cannabinoid levels that were measured at 0, 4, 8, 12 weeks and the completion of the efficacy study of the effects of CBD on osteoarthritis. (40)

6. *AKC Canine Health Foundation study of cannabidiol use for refractory epilepsy in dogs*

The American Kennel Club Canine Health Foundation has granted nearly $400,000 in funding to this research group at CSU for a larger, expanded study with 60 dogs, as a result of the positive results of this pilot work, with respect to the use of CBD oil to address refractory epilepsy. This study will also be looking at uncontrolled epileptics having 2 or more seizures per month while receiving standard therapy. In this expanded study, which will use a cross-over design, each subject will receive 12 weeks of treatment or placebo with a 4-week washout period between treatments. This study began in January 2018, and is currently enrolling patients. (40)

CORNELL UNIVERSITY

The objectives of this recent Cornell study were to determine the oral pharmacokinetics and safety, as well as analgesic efficacy of using CBD in dogs with osteoarthritis (OA). Single-dose pharmacokinetics were performed using two different doses of 2mg/kg and 8 mg/kg of CBD in a carrier oil. From this data, a prospective, randomized, placebo-controlled, double-blind crossover study was conducted using 16 client-owned dogs with radiographically confirmed evidence of osteoarthritis who were enrolled and who completed this study. Dogs were randomized to receive either 2 mg/kg q12h orally of CBD oil, or a placebo consisting of olive oil with a benign herbal extract at a similar volume q 12 h for 4 weeks. Subjects were given a 2-week washout period and then the treatments were crossed-over and each subject received the other treatment twice daily for 4 weeks.

Veterinary assessment of lameness, movement, and response to manipulation, owner questionnaires (Canine Brief Pain Inventory [CBPI], Hudson activity scale), objective kinetic analysis on a pressure-sensitive walkway, hematology and chemistry analysis were obtained at weeks, 0, 2, and 4 for both oils. Statistical analysis was performed on the results, with a $p<0.05$ considered to be significant.

Pharmacokinetics showed a half-life of elimination of 4 - 6 hours at both doses and no observable side-effects. Median maximum concentration of CBD oil was 102 ng/ml (61 - 132 ng/ml) and this peak was reached at 90 minutes following administration of the single dose of 2 mg/kg. The investigators on this study decided that since the pharmacokinetics of the 2 mg and 8 mg doses were so similar they chose to use the lower of the two doses for the efficacy wing of this study.

Assessment of pain and mobility showed a significant decrease in pain and increase in activity ($p<0.001$) at week 2 and 4 during CBD treatment as compared to baseline at each bi- weekly evaluation. It was found that the CBD oil resulted in reduced pain scores when compared to baseline on both bi-weekly examinations ($p = 0.03$). No side effects were reported by owners, but serum chemistry demonstrated an increase in serum alkaline phosphatase (9/16 dogs: 56%) while receiving the CBD oil, which reached significance at week 4 ($p<0.005$).

The authors of this study conclude that the dogs with OA who received 2 mg/kg q 12 hours were found to be more comfortable and active with very few undesirable side-effects compared to placebo. (42).

POTENTIAL AREAS OF FUTURE VETERINARY RESEARCH

Oncology

- Anti-neoplastic activity
- Ameliorate cancer therapy side-effects
- Appetite
- Nausea
- Well-being

Terminal disease – Hospice caregiving

Neurodegenerative conditions

- Spinal stenosis
- GME
- DM

Ophthalmology

- Glaucoma
- Anterior uveitis

Pain Management

- Synergy with other pain meds?
- Condition specific dosing for pain

Behavioral Studies

- Anxiety
- Noise phobias
- Social tension
- Aggression

DOSING RATIONALE FOR VETERINARY CLINICAL USE OF CANNABIS

Dosages in veterinary species have not yet been determined through Phase One tiered dosage studies where clinical response to incremental dosage escalation is measured to determine the optimal dosage for a given condition. In the two university-based studies from Cornell and Colorado State University, pharmacokinetics have been studied at dosages significantly higher than dosages that have been reported through hundreds to thousands of dog and cat administrations by veterinarians who have been recommending hemp based phytocannabinoids prior to Phase One studies, based on effective clinical responses.

The higher dosages used in these pK studies confirmed that plasma levels of cannabidiol are detectable, and persist for sufficient time (5-6 hour half times) to allow for clinical response in conditions of osteoarthritis and refractory epilepsy.

The lower dosages reported to be clinically effective by veterinarians are on an order of 4-5 times lower than the university study dosages. It has been reported in the literature that there is a biphasic dosing response to phytocannabinoids, where lower dosages have one benefit, and higher doses have other benefits. This has been reported in the human literature, but not yet in the veterinary literature. (44) (45) The lower dosage tiers are referred to as “microdosages” and the higher dosing tiers are referred to as “macrodosages”.

Microdoses can be considered to be less than 0.5 mg/kg BID of cannabinoids; macrodoses would be equal or greater than 2.0 mg/kg BID. Individual responses to dosage levels may vary. Based on anecdotal reports by veterinarians and pet owners who are using microdoses, the effective dosages used in dogs and cats have been reported to be as much as 40 times less than the macrodoses used in the CSU safety study or 5 times less than those used in the CSU efficacy study.

EQUINE DOSING STUDY

This author, in an unpublished study, gave 30 horses in three different stables, dosages of 25 – 50 mg of CBD in a zero-THC hemp extract once or twice daily to address complaints of anxiety, gait abnormalities, mild to severe laminitis, and metabolic syndrome. Study subjects averaged around 1000 pounds. It was found that for anxiety and mild cases of lameness or gait abnormalities, administration of 25 mg once or twice daily was adequate to elicit a response with regards to anxiety from loading up into a trailer or at events, or for mild gait abnormalities. In one stable the horses were only able to be given their dose once daily, yet that single dose still produced good clinical results.

For more severe conditions such as moderate laminitis, other sources of lameness, or metabolic syndrome, it was found that 50 mg once or twice daily was sufficient for clinical response. When horse owners were asked to discontinue giving the hemp extracts, so as to determine withdrawal times for CBD for situations in which the horses may be drug tested for an event, many refused to stop administration of the hemp, as they were very pleased with their horses' response to the hemp extracts. Horses have evolved to be very efficient in absorbing fats from their diet, as their natural diet of forage is very low in fats and oils. PK studies in the equine are very much needed to better understand dosing intervals and levels. (46)

POTENTIAL PROBLEMS WITH VETERINARY USE OF PHYTO-CANNABINOIDS

UNWANTED SIDE-EFFECTS

In the macro-dosing safety studies performed by CSU and Cornell, elevations in serum alkaline phosphatase, diarrhea and sedation were observed. These adverse events have been reported, although less frequently with the use of micro-dosing. This same dose-dependent adverse event occurrence has been reported in the human as well. (45)

ADVERSE EVENT REPORT FROM NASC FOR HEMP PRODUCTS

The National Animal Supplement Council (www.NASC.cc) tracks adverse events in nutraceutical and nutritional ingredients submitted by its 175 members and has data on millions of administrations of these ingredients in veterinary species.
The Adverse Event Report (AER) for all forms of hemp in veterinary species has recorded 4,746,313 administrations sold for all forms of hemp to dogs, cats and horses. Since this data has been collected starting in 2010, there have been three adverse events reported in dogs. 2 in 2013, and 1 in 2017. These were not deemed serious adverse events, which would have an incapacitating effect or a non-transient health effect. An adverse event as defined by the NASC is a type of complaint where a patient has suffered any physical effect or health problem that may or may not be connected to or associated with the use of a product. (47)

DRUG-HERB INTERACTIONS

Its reported that in human medical marijuana patients, physicians have only rarely observed clinically significant drug interactions. These two cannabis researchers state, based on their clinical experience and the pooled experience of medical cannabis physicians worldwide: "there is no drug that cannot be used with cannabis, if necessary." (9) Depending on the strength of the affinity of the metabolite for the metabolizing cytochrome, serum levels of cannabinoids or pharmaceuticals may increase with inhibitors or decrease with enzyme inducers. It is known that THC and CBD, in the human, are oxidized by the p450 cytochromes (CYP2C9, 2C19, and 3A4). It is assumed that this is similar to the metabolic pathways in the dog and other veterinary species, but studies still need to be conducted in each species to detail possible inter-species variation.

McGrath (40) in her refractory epilepsy study measured both post-pill phenobarbital levels and potassium bromide (KBR) levels in her study subjects at the end of the 6 week study period. She found no statistical difference between the treatment and placebo groups for either anti-convulsant.

This may indicate that, in spite of the theoretical potential for herb-drug interaction between CBD and P450 metabolized drugs, that at least in dogs on anti-convulsants, the interference may not be clinically relevant. Regardless, it is good medical practice to retest important serum drug levels 2 weeks following initiation of CBD therapy, especially if using macro-dose protocols. McGrath reported that the small study group size in this pilot work may not have given an accurate indication of whether or not there really is herb-drug interaction, and she stated that these levels will be analyzed not just at the end of her AKC-funded clinical trial, but at regular intervals throughout that much larger clinical trial. (40)

WHAT DOES THE FUTURE HOLD FOR VETERINARY CANNABIS?

1. HEMP IN ANIMAL FEED INITIATIVE WITH FDA-CVM AND AAFCO

Current initiative to submit a Feed Additive Petition to the FDA-CVM to have hemp seed approved as an ingredient for dogs, cats and horses.

2. PLANT BREEDING FOR SPECIFIC CULTIVARS

Pharmaceutical companies, such as the UK company GW Pharmaceuticals that has recently had their Epidiolex drug containing CBD approved by the US FDA, have been genetically breeding cannabis plants to increase individual cannabinoids to develop into additional pharmaceutical and OTC products.

3. FDA VETERINARY DRUG APPROVAL FOR PATENTED SYNTHETIC CANNABINOIDS

Several companies are following the FDA track for approval of synthetic cannabinoids as veterinary drugs.

4. CANADIAN FEDERAL LEGALIZATION IS HERE

What does that mean for US/Canadian veterinarians?
The Canadian legislation does not contain language specific enough to allow the use of medical cannabis by veterinarians, but that is currently being addressed by the Canadian veterinary community.

5. GMO CANNABIS IS COMING (GOOD OR BAD?)

This is an inevitable development based on current genetic technology. It remains to be seen if this will be a beneficial development.

CONCLUSION

Cannabis has come a long way since the days of “Reefer Madness”, but there still is much research and clinical work to be done before veterinarians can be 100% comfortable about using this unique and emerging popular therapeutic. The regulatory environment is loosening its restrictions on research and clinical use, and both veterinary clients and veterinarians are eager to get involved.

University studies have shown safety and efficacy at macro-dosing levels, and NASC adverse event reports and field reports from veterinarians and their clients indicate similar safety and efficacy at lower doses. Herb-drug interactions are a concern, given cannabidiol’s metabolic disposition through the P450 cytochrome system, but clinically reports indicate that does not seem to be a problem.

FDA-approved cannabis drugs for humans are on the market, and similar approval for veterinary labeled drugs can not be far off. Similarly, genetic breeding of cannabis cultivars for product development will improve the quality of veterinary and human drugs and cannabis nutraceuticals, and genetic engineering of the cannabis genome is awaiting Federal legalization.

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