PhD THESIS

Safety studies and biological effects of a cannabidiol-based product

(SUMMARY OF THE PhD THESIS)

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INTRODUCTION

Cannabidiol (CBD) is one of the non-psychoactive phytocannabinoids extracted from the *Cannabis spp* plant. The lesser known phytocannabinoid, especially when compared to the much more popular, and much more restricted delta 9-tetrahydrocannabinol (THC), does not appear to provoke any dependence or any other similar side effects. This is mainly due to the fact that CBD does not seem to directly activate receptors CB_1 and CB_2, which is the main mechanism of action of THC (PERTWEE et al., 2004).

In recent years, cannabidiol has gained more and more traction worldwide as a stand-alone or complementary therapeutic molecule in a vast array of pathologies. As there is some controversy pertaining to CBD and its uses, in-depth studies, both concerning safety of the product as well as its uses in various pathologies have not been conducted. Furthermore, since cannabidiol products are not legal in all countries or territories around the world, research on CBD is not as easily done. The United States of America's FDA has approved one single cannabidiol formulation, Epidiolex, and it has been approved for use in childhood refractory epilepsy pathologies such as Lennox-Gastaut Syndrome and Dravet syndrome (FASINU et al., 2016). Europe's regulatory body, EMA, has given CBD (Epidyolex) the designation of "orphan medicine" for the treatment of Lennox-Gastaut Syndrome, Dravet syndrome and tuberous sclerosis. For any other pathologies, CBD has not been approved and therefore is considered a supplement, not medicine and thusly is much less regulated than conventional pharmaceutical preparations. Numerous preliminary studies have been conducted researching other potential benefits of cannabidiol, which will be discussed later in this thesis.

The main problems in both human and veterinary medicine concerning furthering knowledge vis-à-vis the use of CBD are divided. The chief issue with CBD is that many people simply do not know the difference between CBD and THC, and believe that they are the same thing, which is equivocally wrong. They are two very different molecules with very different effects and targets. Both can be used in the scope of medicine, however the psychoactivity of the THC molecule is what people are weary of. Strict worldwide laws and regulations concerning the use of THC and inadvertently, CBD products also hinder further research into the subject of CBD. Slowly, as countries differentiate between the two molecules, more research into CBD has been conducted and more can be expected. In human medicine, CBD is mostly used for the conditions mentioned above with other "off-label" uses which will be enumerated in this thesis. In veterinary medicine, the use of CBD is much more withheld, with many practitioners hesitant to advise its use. The United States of America and Canada are at the forefront

of CBD use in pets, mainly in dogs. A handful of small, successful clinical studies in dogs have highlighted the utility of CBD as a standalone or combination therapy in epilepsy and osteoarthritis (MCGRATH et al., 2019; GAMBLE et al., 2018; MEIJA et al., 2021; LISA MORROW et al., 2020; FREDERICA BRIOSCHI et al., 2020; GARCIA et al., 2022; HEIDRUN POTSCHKA et al., 2022; ANDRA POPESCU et al., 2022; MOGI et al., 2019),
however, the data in cats is quite scarce.

As laws become less strict, pet owners are more and more willing to explore CBD as a potential therapeutic agent for their companion animals, and perceptions of the owners are becoming more open to the idea. A survey was conducted by Kogan et al in the United States, and found that less than half of practicing veterinarians felt comfortable suggesting CBD to owners of their patients. Moreover, younger veterinarians were more likely to prescribe CBD mainly for pain management and epilepsy than older veterinarians. Participants voiced that the veterinary regulatory bodies could provide more support with regards to the use of veterinary cannabidiol, especially because the laws are not yet clear on the use of CBD (LORI KOGAN et al., 2019). A similar study was carried out in Slovakia; however, it was meant to gauge owner's perspectives on CBD. 38.5% of respondents have already or were currently giving CBD to their pets and most of them received their information regarding CBD from the internet with only 34% of people receiving information from their veterinarians. More than half of the participants in this study stated that they were using CBD as a conjunctive treatment (KATERINA TOMSIC et al., 2022).

Most veterinarians agree that there is nowhere near sufficient enough research into veterinary CBD use, and more studies are needed in order for practitioners to feel safe prescribing CBD in animals.

As laws change, more research occurs and CBD products become more readily available, it will be easier for veterinarians to access information and be able to use cannabidiol in conjunction with conventional treatments in various pathologies for dogs and cats.

AIMS AND OBJECTIVES OF THE RESEARCH

The **working hypothesis** is based on the assumption that the cannabidiol molecule possesses anti-epileptic and neuroprotective effects, which may lead to its concomitant use in treating canine epilepsy and in chemotherapy-induced peripheral neuropathy in both animals and humans.

The **aim** of this research is to test the safety of a Romanian, highly concentrated cannabidiol product, to assess its potential therapeutic effects in canine epilepsy and to see whether or not the molecule is able to mitigate the effects of vincristine-induced peripheral neuropathy.

Objective 1

The main objective of the first study is to assess the acute safety of a highly concentrated cannabidiol product. This is to be done by means of an acute toxicity study as per OECD protocols, complete with biochemical, histological and hematological analyses. Furthermore, a complete characterization of the product is to be done, alongside a heavy metal analysis.

Objective 2

The main objective of the second study is to assess the effectiveness of cannabidiol in mitigating the severity of vincristine induced peripheral neuropathy. The specific objectives of this study include behavioral analysis of the rats by means of the Rat Grimace Scale, Tail Flick test and pain perception tests.

Objective 3

The main objective of the third study is to evaluate the efficacy of cannabidiol as a concurrent treatment of epilepsy and osteoarthritis in dogs. The efficacy of the molecule will be evaluated with radiological, hematological and biochemical analyses as well as pain evaluations with the Colorado Pain Scale.

STRUCTURE OF THE DOCTORAL THESIS

The thesis entitled "Safety studies and biological effects of a cannabidiol-based product" consists of 124 pages written according to current editing norms at both the academic and national level. The doctoral thesis consists of two parts comprising 8 chapters and contains 44 figures, 19 tables and 208 bibliographical sources.

The **first part** of the doctoral thesis entitled "Literature Review" is structured in 3 chapters and covers 43 pages. This part encompasses information relating to cannabidiol with its synthesis pathways, and various phytocannabinoids synthesized from the *C. sativa* plant (**Chapter 1**). It also includes the presentation of the endocannabinoid system with the endocannabinoids and the CB_1 and CB_2 receptors and their interactions within the body, as well as the pharmacokinetics and pharmacodynamics of cannabidiol. The next chapter comprises the effects of cannabidiol in both human and veterinary medicine alongside safety data for the CBD molecule (**Chapter 2**), while the last chapter consists of information regarding vincristine and chemotherapy-induced peripheral neuropathy (**Chapter 3**).

The **second part** of this doctoral thesis comprises original research and consists of 5 chapters spanning 80 pages.

The beginning of the second part of the thesis offers information regarding the main aims and the working hypothesis of the thesis as well as the objectives for each of the following studies. The aim of this research is to test the safety of a Romanian, highly concentrated cannabidiol product, to assess its potential therapeutic effects in canine epilepsy and to see whether or not the molecule is able to mitigate the effects of vincristine-induced peripheral neuropathy.

Chapter 4 contains **Study I**: Characterization, Heavy Metal Analysis and Acute Toxicity Study of Cannabidiol and the main goal of this study was to characterize the phytocannabinoids and their levels in the CBD oil, to assess the presence of heavy metals in the product and to perform a 14-day acute toxicity study of the product on rats. The toxicity study included monitoring hematological and biochemical parameters, weight fluctuations during the study period and histopathology at the end of the study.

Chapter 5 contains **Study II**: Cannabidiol in the mitigation of Vincristine-Induced Peripheral Neuropathy and the main objective of this study was to assess the effectiveness of cannabidiol in mitigating the severity of vincristine-induced peripheral neuropathy. Various tests were conducted throughout the experiment to evaluate the effects of CBD on VIPN, including behavioral tests (Tail Flick Test, Paw Withdrawal Test), histopathology and immunohistochemistry, hematology and biochemistry. The Rat Grimace Scale was also utilized in order to monitor pain throughout the experiment.

Chapter 6 includes **Study III**: Cannabidiol in the Management of Canine

Epilepsy and Arthritis. The main objective of this study was to evaluate the efficacy of cannabidiol as a concurrent treatment of epilepsy in dogs. Since the dog was also diagnosed with osteoarthritis, monitoring any changes in pain related to the arthritis as well as the progression of the disease were also done. The patient was monitored daily throughout the experiment by means of the Colorado Pain Scale and paraclinical investigations (hematology, biochemistry and radiology) were conducted every 6 months.

Chapter 7 presents general conclusions and recommendations based on the results of the 3 studies conducted for this doctoral thesis. **Chapter 8** summarizes the originality and innovative contributions of the thesis.

RESULTS OF THE ORIGINAL RESEARCH

Chapter 4. The aim of our acute toxicology study was to confirm or deny the safety of use of the CBD full spectrum product. This study is conclusive that this highly concentrated CBD product does not show acute *in vitro* or *in vivo* cytotoxicity, even at very large doses. The studies on cell cultures showed both viability and cell proliferation.

The post-mortem histopathological study carried out on the liver and kidneys as well as other organs of the various subjects did not show the presence of either storage or morpho-functional alterations of the necrotic type, among others. The safety of the CBD product regardless of the dose, without association with the appearance of undesirable side effects, or mortality in the various study subjects was observed in this study. It was also noted that 100% of the subjects gained weight without any change in clinical status or behavioural parameters.

No mortality was revealed, including at the maximal dose of 2000mg / kg, no toxicity at the organ level was demonstrated by our study at this dosage, indicating relatively high safety of *in vivo* use of the product.

Chapter 5. This study aimed to assess the effectiveness of cannabidiol in mitigating the severity of vincristine-induced peripheral neuropathy.

In terms of the results obtained, it does appear that, under the circumstances, CBD confers a certain level of neuroprotection to rats during vincristine administrations. The Vin+CBD group showed the least amount of weight loss, and statistically, the least amount of pain (P=0.049), faster reflexes for the paw withdrawal test (P=0.021) and a faster reaction time to the cold ethanol (P=0.030) than the VIN and Vin+GABA groups.

With regards to body weight, weight loss was expected during the course of this experiment, due to the vincristine administration, as it is a common side effect. Cannabidiol reduced the amount of weight loss in the experimental group, in comparison to the groups that received vincristine and gabapentin and only vincristine.

For the behavioral tests conducted within this experiment, cannabidiol does ameliorate the effects of vincristine, in comparison with the other 2 experimental groups. These findings are similar to what has been published in preliminary studies, as CBD is a neuroprotective substance, however the mechanisms implicated are not yet clear.

Chapter 6. This study aimed to evaluate the efficacy of cannabidiol as a concurrent treatment of epilepsy and osteoarthritis in dogs. Our patient had epileptic seizures about 3 times in the first month of CBD treatment, although she was on a daily therapy with phenobarbital and phenytoin at a dose of 100 mg / animal, each. After the first month of administration of CBD, in addition to her therapy with phenobarbital and phenytoin, the seizures were noticeably reduced, in frequency and severity. At the same time, after 6 months of treatment, the improvement of certain biochemical values, back within normal limits is observed. The administration of CBD has led to a decrease in epileptiform seizures and to the relief of pain and muscle contractions associated with the epilepsy pathology, thus avoiding the destruction of muscle tissue, evidenced by a decrease in the enzyme creatine phosphokinase.

After the last crisis observed in the first month of CBD administration, no more seizures were recorded over an 8-month period. After 4 months of clinical "tranquility" of epilepsy, phenytoin was removed from the treatment, since the hepatotoxicity of this compound is well known. After another 3 months without the presence of epilepsy symptomatology, phenobarbital was removed from the dog's therapy as well. These actions were felt, in the sense that on the biochemical analysis performed 3 months after the elimination of phenobarbital, all of the biochemical parameters came to be within normal limits, and due to the effects of myorelaxant and analgesic CBD, including the muscle injury seen on the first biochemical analyses, was not found in the analyses performed later. Also, two mild epileptic seizures, short in duration, were observed, one month and two months, respectively, after the elimination of phenobarbital from therapy. These could also be attributed to the fact that the animal had previously had a 4-day "window" during which it was not given any anti-convulsive therapy, due to a problem in transporting the CBD product. Another 5 months of lack of seizure activity followed, punctuated by a mild epileptic seizure synchronous with the onset of heat, after which it was decided to increase the dose of CBD to 4 mg / kg body weight. Since then, no further epileptic seizures have been recorded until the end of the study.

GENERAL CONCLUSIONS

Chapter 7 of the doctoral thesis states the general conclusions reached as a result of the current research project and are as follows:

1. The particular cannabidiol product produced by SEVA SRL, is not contaminated with heavy metals, and the characterization matches exactly what is stated on the label.
2. Cannabidiol is relatively safe, and was well tolerated by rats, even in very high doses. It does not produce *in vitro* or *in vivo* toxicity acutely in rats. *In vitro* experiments on cell cultures even show cell proliferation at higher concentrations.
3. Cannabidiol shows a great degree of neuroprotection in a chemotherapy-induced peripheral neuropathy model in rats, lessening pain and the degree of hypoalgesia. Furthermore, cannabidiol lessened the inability to feel cold temperatures and caused less weight loss in the rats.
4. Cannabidiol provides very good anti-epileptic effects in dogs, and acts as an analgesic in chronic pain caused by osteoarthritis, however it cannot stop the natural progression of osteoarthritis in older animals.
5. This cannabidiol product did not increase biochemical parameters, especially AST and ALT after 17 months of daily use, contradictory to what some previously published articles have stated.

RECOMMENDATIONS

Overall, my recommendations with regards to the use of cannabidiol in veterinary medicine for various pathologies including epilepsy and osteoarthritis, are as follows:

Cannabidiol is effective and can be used as a conjunctive treatment or a stand-alone treatment in certain cases of epilepsy in dogs, after slowly weaning the animal off of conventional anti-epileptic medications. The starting dose should be 2-2.5mg/kg and then it can be titrated up if the need arises to 5-6mg/kg.

Cannabidiol is effective and can be used in canine osteoarthritis, where it acts as both an analgesic and an anti-inflammatory agent.

Cannabidiol can ameliorate the severity of vincristine-induced peripheral neuropathy, and may be more useful as a conjunctive treatment in chemotherapy protocols mainly in human medicine rather than veterinary medicine, as the prevalence of VIPN in veterinary medicine is low.

It is difficult to say with certainty that all cannabidiol products available on the market are created equally, as earlier studies have shown that the lack of regulation of plant-derived nutraceuticals lead to mislabelling and misrepresentation.

Further studies are needed to investigate the beneficial effects of cannabidiol in cats, as there is a severe deficiency of research in cats. Further studies in both cats and dogs are needed in order to investigate further benefits of CBD in felines and canines.

In terms of the neuropathy study, my recommendation is that the study be conducted during a chronic period of time due to the fact that cannabidiol is a plant derived product and requires a longer period of time to accumulate in the system and provide therapeutic effects.

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