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Cannabinoid Modulation Of Cisplatin Induced Neuropathy

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CANNABINOID MODULATION OF CISPLATIN INDUCED NEUROPATHY

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ABSTRACT

Endocannabinoid modulation of cancer-related pain is well-documented. Sativex, a cannabinoid extract with a 1:1 ratio of tetrahydrocannabinol (THC) and cannabidiol (CBD) has been shown to alleviate neuropathic pain associated with chemotherapy. This research examined whether THC or CBD alone is effective in attenuating or preventing tactile allodynia associated with cisplatin-administration. Mice (C57BL/6) were given eight doses of 2.3 mg/kg cisplatin or saline solution IP every second day to induce tactile allodynia (Ringers on alternate days). Tactile responses to hind-paws were quantified in g of force using an electric von Frey (eVF) prior to (baseline) and after the cisplatin administration protocol. Separate groups of mice were then given vehicle, 100 mg/kg gabapentin, 2 mg/kg THC or 2 mg/kg CBD IP and tested 60 m later on eVF. In the prevention studies, CBD (0.0, 0.5, 1.0, and 2.0 mg/kg) or THC (0.0, 0.5,1.0, and 2.0 mg/kg) was given IP 30 m prior to cisplatin administration (2.3 or 1.0 mg/kg) utilizing a six-dose alternate day protocol. As before, tactile response was measured using eVF prior to and after cisplatin dosings. Cisplatin produced a reduction in g of force indicative of neuropathy in each study. Gabapentin, THC, and CBD did not alter tactile responses in control mice. Cisplatin allodynia was attenuated by gabapentin, THC, and CBD but was not prevented by either cannabinoid tested. These data demonstrate that THC and CBD administered alone, unlike that in Sativex, can achieve analgesic effects in this murine model of cisplatin neuropathy.

DEDICATION

I would like to dedicate my thesis work to my family and friends. I am beyond grateful for my mother's words of wisdom, my father's tough love, and the support shown by my sisters- Dana, Emma, and Jill and by my brother Michael. I wish to express a sincere thank you to my grandparents for their encouragement throughout my studies and their unconditional love. I am also thankful for Ivy, you have been with me since the beginning of this project and I am deeply thankful for your patience and support.

I would also like to dedicate my thesis to my biggest fan and best friend of 6 years, Bella Roo Harris.

I am tremendously fortunate to have you all in my life and cannot thank you all enough for all that you have done.

LIST OF ABBREVIATIONS AND SYMBOLS

CIPN	Chemotherapy-induced peripheral neuropathy
DNA	Deoxyribonucleic acid
DRG	Dorsal root ganglia
CIN	Cisplatin induced neuropathy
TCA	Tricyclic antidepressants
SSRI	Selective serotonin reuptake inhibitors
ES	Endocannabinoid system
AEA	N-arachidonylethanolamine, Anandamide
2-AG	2-arachidonoylglycerol
CB	Cannabinoid
CNS	Central nervous System
PNS	Peripheral nervous System
EPIS	Endogenous pain inhibitory system
PAG	Periaqueductal gray
GABA	Gamma-aminobutyric
THC	Δ^9-tetrahydrocannabinol
CBD	Cannabidiol
BBB	Blood brain barrier

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CHAPTER 1

INTRODUCTION

Cancer is an uncontrolled proliferation of abnormal cells that spread and invade body tissue (Funk & Wagnalls). No single mechanism leads to the development of cancer. Numerous external factors, such as tobacco and infectious agents (e.g., pesticides, herbicides), and internal factors, such as genetic mutations, all contribute to the development of cancer (Erenler, 2014; Cancer.org). Currently, 200 types of cancers have been identified and are classified by the type of cell or origin and the tissue it invades. These various cancer types form three subcategories; sarcomas, carcinomas, leukemias/lymphomas. Treatment of cancer includes surgery, radiation, immunotherapy, and chemotherapy.

Chemotherapy is an effective and commonly used oncology treatment (Galmarini et al., 2012). Types of chemotherapeutics include antimetabolites, anti-tumor antibiotics, mitotic inhibitors, and alkylating agents and are assigned based on cancer types and stages of cancer progression. Chemotherapy drugs stop the spread, slow the growth, and/or kill cancer cells by preventing these cells from reproducing. However, chemotherapy drugs cannot differentiate cancerous cells from healthy cells, resulting in destruction of healthy cells. The destruction of healthy cells produces unwanted toxic side effects and limits the usefulness of chemotherapy. Common side effects of chemotherapy ilude nausea, hair loss, and suppression of immune systems. The side effect of unrelenting chemotherapy related pain is a significant oncology

problem and a feared consequence of treating cancer with chemotherapy (Valeberg et al., 2007). Studies reveal that approximately 3.5 million oncology patients suffer from chemotherapy related pain daily leading to a diminished quality of life (Windeback and Grisold, 2008; Paice, Kumar, 2011).

The most prevalent type of oncology pain is chemotherapy-induced peripheral neuropathy (CIPN) with incidence rates ranging from 19% to more than 85% (Fallon, 2013).Of the chemotherapy classes mentioned above, taxanes, vinca alkaloids, and platinum compounds lead to the development of CIPN (Kannarkat, 2007). Unfortunately, CIPN does not abate immediately after chemotherapy. A current meta-analysis of 4,179 patients showed CIPN was prevalent in 68% of patients one month post chemotherapy, 60% at three months, and 30% at six months (Sertny et al., 2014). CIPN is often the reason for reduction and discontinuation of chemotherapy (Carozzi et al., 2014). Long-term management of pain is therefore becoming one of the most challenging aspects of treatment for neurologists and oncologists, thus the need for palliative care in treating chemotherapy side effects is of great importance (Windebank and Grisold, 2008).

CHAPTER 2

BACKGROUND

Cis-diamminedichloroplatinum (II), commonly called cisplatin, is a platinum chemotherapy frequently used in oncology treatment. Approved in 1978 by the Food and Drug Administration, to this day it is a mainstay in oncology treatment. Cisplatin treats a wide variety of cancers and is referred to as "the penicillin of cancers" (cisplatin.org). Cisplatin is used as standard therapy in many pediatric cancers and is the first line of defense in treating sarcomas, testicular and lung cancers (Dasari et al., 2014). Perhaps the most successful results of cisplatin are seen in treating testicular cancer where cisplatin alone has decreased mortality from 90% at 5 years to a cure rate of more than 80% (Windebank and Grisold, 2008). While cisplatin is effective as a treatment alone, it is has also shown success in combination therapy to treat chemotherapy resistant cancers (Dasari et al., 2014)

Cisplatin is administered intravenously and becomes active when it diffuses into a cell. The main mechanism of action is oxidative stress that leads to cell apoptosis. Oxidative stress is an imbalance of the cell's biological functions impairing them to protect or repair damage. Cells are chemically reactive molecules that regulate homeostatic oxygen levels. When oxygen levels are no longer in a homeostatic state, cellular damage occurs to proteins, mitochondria, and deoxyribonucleic acid (DNA). Cancer cells display robust reactions to oxidative stress leaving them vulnerable to mitochondria malfunctions. Cisplatin capitalizes on this and directs oxidative stress specifically to the mitochondria of cells causing an imbalance of oxygen and other

biological responses resulting in apoptosis. This specific mechanism of action makes cisplatin a potent antitumor agent. However, cisplatin is not without its shortcomings. Because cisplatin damages DNA, many toxic effects are produced leaving patients with a number of side effects that can limit its use. Cisplatin's side effect profile includes cardiotoxicity, ototoxicity, and neurotoxicity (Florea, 2011).

Therapeutic doses of cisplatin cannot cross the blood brain barrier resulting in toxicity to peripheral tissues, notably the dorsal root ganglia (DRG) (the location of cell bodies of sensory neurons) and sensory nerve fibers (Gregg et al., 1992). Cisplatin damages sensory fibers, deteriorates the volume of nerve fibers, and impairs the function of myelin sheath. These damages decrease the conduction velocity of action potentials in nerve pathways resulting in chronic neuropathic pain known as cisplatin induced neuropathy (CIN) (Carozzi and Chiorazzi, 2014).

Cisplatin has a dose-limiting effect where 50-85% of patients develop CIN three to six months into treatment from the cumulative dose of 400-500mg/ m^2 (Paice, 2010;Windeback, 2008; Amptoulach and Tsavaris, 2011). CIN presents in distal portions of limbs in a "stocking and glove" distribution causing paresthesia, hyperalgesia, and allodynia (Paice, 2010). Hyperalgesia presents as an increased response to a stimulus that is normally painful and allodynia presents as pain due to a stimulus that does not normally activate the nociceptive system (Taverner, 2014). CIN is irreversible in 30-50% of patients. Unique to platinum compounds like cisplatin, neuropathic pain does not cease after treatment. A phenomenon known as "coasting" is produced where neuropathy can persist several months to years after ending cisplatin treatment (Grisold et al., 2012). A 2002 follow up study of testicular cancer survivors revealed 55% of patients had residual CIN pain 15 years after treatment (Strumberg et al., 2002).

Current CIN treatment includes off- label usage of tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRI), anticonvulsants, and opioids (Trivedi et al., 2013). Sadly, randomized controlled trials of TCAs, SSRIs, and anticonvulsants show poor attenuation of CIN pain and adverse side effects where 30% of patients discontinue treatment (Trievedi et al., 2013; Hammack et al., 2002; Kautio et al, 2008; Rao et al., 2008; Rao et al., 2007). The anticonvulsant Gabapentin is often used as a gold standard in treating CIN pain because of its low side effect profile (Reardon and Saif, 2005) Opioids are found effective but their use is limited due to a harsh side effect profile affecting 76-96% of patients. Often neuropathic pain becomes intolerable and cisplatin doses may be decreased or discontinued jeopardizing a patient's chance of survival. Preventing the onset of CIN in patients undergoing treatment is major goal in oncology but has proven unsuccessful in clinical trials due to flawed study designs and small sample sizes. Treatment should not only show efficacy in relieving symptoms but also avoid hindering the antitumor effect of cisplatin, making novel analgesic treatment a challenge in developing new pharmacotherapies (Paice, 2011). Because CIN is a treatment-limiting side effect of cisplatin, it is critical to develop novel approaches for the treatment and prevention of CIN.

The endocannabinoid system (ES) mediates many physiological functions such as movement, mood, and nociception. The ES contains endogenous ligands anadamide (N-arachidonylethanolamine, AEA) and 2-arachidonoylglycerol (2-AG) along with G-coupled cannabinoid (CB) CB1 and CB2 receptors. These receptors are expressed on many nociceptive areas in the central nervous system (CNS) and the peripheral nervous system (PNS). CB1 receptors are highly expressed in the CNS and moderately in the PNS. CB1 receptors modulate nociception by activation of the endogenous pain inhibitory system (EPIS). These pain

regulating sites include the periaqueductal gray (PAG), thalamus, amygdala, spinal cord as well as the DRG. CB2 receptors are expressed on immune cells in the periphery and on EPIS sites in the CNS that includes the DRG, spinal cord, and PAG. CB2 receptors in the spinal cord are inducible in neuronal injury and inflammation (Sagar et al., 2005). These receptors are known to mediate inflammatory pain by releasing anti-inflammatory factors and secreting pro-inflammatory factors such as cytokines, chemotaxis and antigen processing of microglia in the CNS and macrophages in the immune system (Rahn and Hohmann, 2009; Chiou; Zogopoulos et al., 2008; Pertwee, 2001).

Cannabis sativa (marijuana) has been used for more than four centuries to treat a variety of medical conditions (Chiou; Amar et al., 2006). In 1839 William O'Shaughnessy published anecdotal observations and reports of cannabis use as an analgesic (Amar et al. 2006; O'Shaughnessy, 1839). O'Shaughnessy's publication began the widespread use of cannabis for medicinal uses.

The two main constituents of cannabis are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). These cannabinoids exert their analgesic effects by an action in the brain via descending modulation, by a direct spinal activation on CB1 receptors, and/or by an action on CB1 and CB2 receptors located on peripheral nerves (Chiou et al., 2013; Zogopoulos et al., 2008). THC, the primary psychoactive constituent in cannabis, is highly lipid soluble and readily diffuses into the blood brain barrier (BBB), binding to CB1 and CB2 receptors effecting sites of nociception that process and encode harmful stimuli. CBD lacks psychoactive properties and has a limited affinity in binding to either receptor and is known to play a role in immune responses as well as nociception (Ameri, 1998;Turo J. Nurmikko; Rahn and Hohmann, 2009;

Chiou). When administered systemically, cannabinoids are known to produce analgesic properties comparable to opioids in acute pain models (Chiou et al., 2013; Walker et al., 2001).

The anti-nociceptive effects of cannabinoids are demonstrated in a variety of rodent pain assays. Administration of synthetic and endogenous cannabinoids produces anti-nociceptive effects in models of acute and chronic pain (Drew et al., 2000). Carrageenan models of acute inflammatory pain show administration of the natural ligand AEA produces a reversal of thermal hyperalgesia (Richardson et al., 1998). Other acute pain models such as the formalin test demonstrate administration of CB1 and CB2 agonist reduces formalin-evoked pain but the administration of CB1 and CB2 antagonist can enhance mechanical paw withdrawal in formalin induced nociception. These studies indicate that CB1 and CB2 receptors mediate tonically inhibitory action on formalin-induced inflammatory pain (Calignano et al., 1998; Wang et al., 2015). Further, the use of cannabinoids in chronic pain assays also demonstrates a significant modulation of pain. Martin et al. demonstrated that administration of CB agonist reverses mechanical allodynia in Freunds's adjuvant rodent model (1999). Animal models of diabetic induced neuropathy have shown that cannabinoid agonists are successful in alleviating mechanical allodynia in type1 and 2 diabetes (Vera et al., 2012). Collectively, these studies offer the use of cannabinoids as an alternative and effective treatment of CIN.

Targeting the ES with synthetic and endogenous cannabinoids has shown to provide analgesic effects in treating CIN. Rodent models of cisplatin neuropathy have demonstrated that elevating endocannabinoid tone through administration of AEA or through inhibiting the enzyme that degrades AEA can attenuate mechanical and thermal allodynia as well as hyperalgesia (Guindon et al., 2012). Further research using the selective CB1 agonist ACEA, the CB2 agonist JWH133, and the non-selective CB agonist WIN 55,212-2 in treating rats with CIN resulted in

attenuation of thermal and mechanical allodynia. While synthetic cannabinoids have shown to attenuate CIN pain there is also a literature showing the same effect can be accomplished through natural cannabinoids.

Recent clinical trials have studied the effects of combining THC and CBD in combination in the treatment of neuropathic pain. Sativex®, a whole-plant cannabis extract, contains a 1:1 ratio of THC and CBD (2.7 mg: 2.5mg) and is administered through an oral mucosa spray. Sativex® is approved in Canada and the UK to treat multiple sclerosis, neuropathic and opioid-resistant cancer pain. Clinical trials of Sativex have shown it to be effective in treating allodynia associated with neuropathy (Nurmikko, Vann). It is unknown if THC and CBD are needed in combination to treat CIN. It is also unknown if THC and CBD can prevent the onset of CIN. The present study seeks to identify if THC or CBD alone are effective in alleviating neuropathic pain characterized by allodynia when induced by cisplatin and if THC and CBD can prevent the onset of CIN.

CHAPTER 3

METHODS

Male C57BL/6 mice (25-30 g) were used in these studies. Food and water were provided ad libitum. Lights operated on a 12:12 hr light dark cycle with lights on at 06:00. Mice were housed 2 per polycarbonate tub with soft bedding. Mice used in Experiment 1 were experimentally naïve. Mice in Experiment 2 were enrolled in an earlier experiment and served as a control group (i.e., non-cisplatin treatment) in a CIN study. These mice had received two exposures to either saline, gabapentin, THC or CBD but were given 1-week drug wash-out before being enrolled in this experiment.

An electronic von Frey (eVF; Topcat Metrology Ltd; Little Downham, UK) was used to measure paw withdrawal thresholds to a mechanical stimulus and determined the presence of tactile allodynia in cisplatin-treated mice. Mice were acclimated in a rectangular enclosure (3.81x11.43x11.43cm) with metal rod floors for at least 5 min before testing. A von Frey filament was applied to the mid plantar region of the hind paw and withdrawal thresholds were recorded. Filaments were applied to alternating left and right hind paws at 3 min intervals for a total of 4 measurements per paw. The average score of these 8 tests served as the dependent measure.

Procedure

Experiment 1

Mice were randomly assigned to receive 9 intraperitoneal (IP) injections of either cisplatin (2.3mg/kg/ml) or saline every other day with lactated Ringer's solution (1.0 ml/kg) on intervening days. Baseline eVF measures were taken several hours before enrollment in the study (Day 1) and revealed balanced group assignment. eVF measurements were also performed on D6, 12, and 18 to assess the presence of tactile allodynia. By D18 (Ringer's solution), cisplatin mice showed significantly lower paw withdrawal thresholds (i.e., allodynia) than the controls. On D19, pair-housed mice were separated in their home tubs via a metal divider and block assigned into saline, gabapentin (100 mg/kg), THC (2mg/kg) or CBD (2mg/kg) groups (n = 4-5). Mice were given test articles on D20 and 21. Dependent measures were assessed 1 hr after IP administration of test articles. To accommodate eVF testing for all mice, half were tested on D20 while the other on D21.

Experiment 2 & 3

Separate cohorts of mice were randomly assigned into either CBD (Exp 2: 0.0, 0.5, 1.0 and 2.0 mg/kg) or THC (Exp 3: 0.0, 0.5, 1.0 and 2.0 mg/kg) treatment conditions (n = 3-4). Baseline eVF measures were taken several hours before enrollment in the study (Day 1) and revealed balanced group assignment. In contrast to Experiment 1, mice received 6 IP injections of cisplatin (Exp 2: 2.3 mg/kg/ml or Exp 3: 1.0 mg/kg/ml) every other day with lactated Ringers solution of intervening days. Further, cannabinoid test articles were given IP 30 min prior to cisplatin administration in an attempt prevent the development of allodynia associated with CIN. eVF measurements were collected under drug-free conditions on D5, 9, and 13.

Statistical Analysis

Data were screened for outliers before data analyses. This included excluding animals that lost more than 25% of original body weight. Animals that lost >25% of body weight were removed from the study and humanely euthanized These procedures were approved by the Institutional Animal Care and Use Committee at the University of Mississippi (Protocol #13-017).The total number of animals omitted based on this criteria equaled 1 from Experiment 1.

Experiment 1

For analyses of induced tactile allodynia, average eVF measurements for allodynia in non-cisplatin and cisplatin animal groups were conducted using a one-way analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) post-hoc tests. A statistically significant decrease in mean withdrawal threshold (g) was considered neuropathic. Sample sizes in each treatment consisted of n = 16-18.

To highlight drug efficacy on tactile allodynia, eVF measurements were analyzed by a two way ANOVA followed by Fisher's LSD post-hoc tests. A statistically significant increase of mean withdrawal threshold (g) compared to cisplatin animals receiving vehicle was considered to attenuate tactile allodynia. Drug sample sizes consisted of n = 4-5.

Experiment 2 & 3

For analyses of preventative effects of CBD and THC on tactile allodynia, average eVF measurements for allodynia under all doses were conducted using a 1-way ANOVA. Statistically significant eVF measurements were followed by Fisher's LSD post-hoc tests. Sample sizes of n= 3-4.

All analyses were considered statistically significance at a level of $p < .05$.

CHAPTER 4

RESULTS

Experiment 1

The effects of cisplatin on tactile allodynia are summarized in Figure 1. Paw withdrawal thresholds in cisplatin mice were lower than non-cisplatin mice and illustrate tactile allodynia associated with neuropathy. A one-way ANOVA on these data revealed a significant treatment effect, $F(1,29) = 4.972$, $p = 0.034$, with mean withdrawal threshold in the cisplatin group significantly lower than non-cisplatin controls.

Figure 1: Effects of Cisplatin on Tactile Allodynia

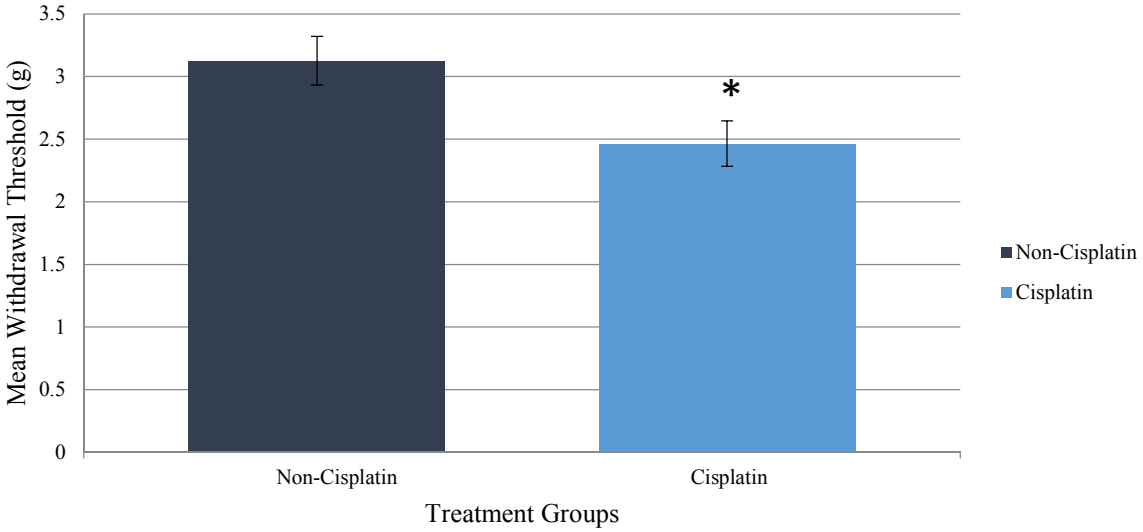


Fig 1. eVF measurements quantified on day 18. Values represent ± SE (n=16-18). * denotes significant difference of withdrawal threshold compared to non-cisplatin group ($p<0.05$).

The effects of gabapentin, THC and CBD on paw withdrawal thresholds across cisplatin treatment conditions are summarized in Figure 2. In general, non-cisplatin-treated groups had higher paw withdrawal thresholds than cisplatin groups. In non-cisplatin-treated mice, gabapentin, THC, and CBD did not alter paw withdrawal thresholds. In the vehicle group, cisplatin produced tactile allodynia (i.e., lowered thresholds). This allodynic response was attenuated by all three test articles. Consistent with these observations, a two-way ANOVA revealed a significant main effect for drug treatment, $F(3,23)=4.08$, $p = 0.018$, and a significant cisplatin by drug interaction $F(3,23)= 3.24$, $p=0.40$ The main effect for cisplatin treatment was not significant $F(1,23)=1.99$, $p = 0.17$. These findings prompted a simple effects analysis for drug treatment in both the cisplatin and non-cisplatin groups; a main effect for drug treatment was found for cisplatin groups ($F(3,12) = 6.10$, $p = 0.009$) but not in the controls. Among the cisplatin groups, mean paw thresholds were significantly higher in gabapentin ($p = 0.001$), THC ($p = 0.010$) and CBD ($p = 0.010$)-treated mice compared to saline mice.

Figure 2: Drug Probes on Tactile Allodynia

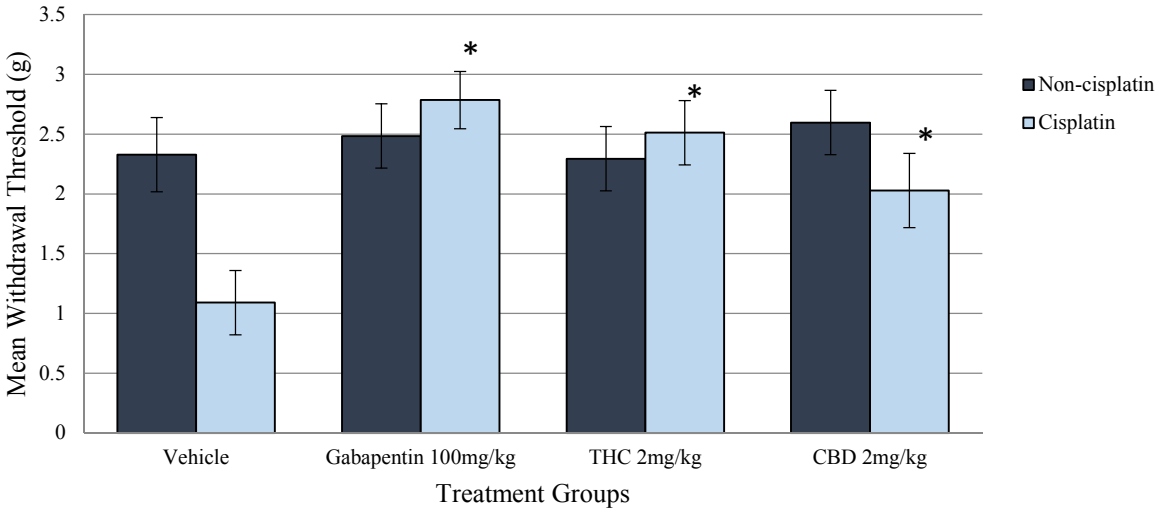
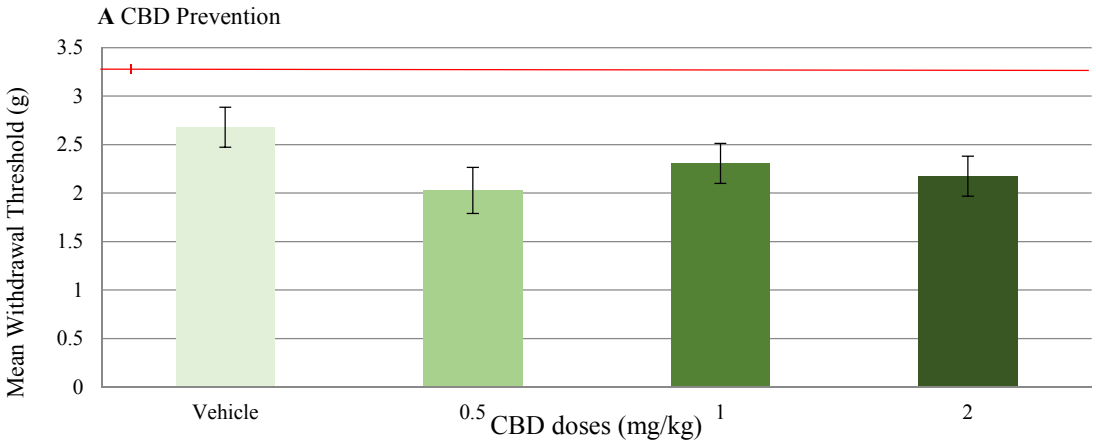


Fig 2. Drug treatment effects of tactile allodynia on non-cisplatin and cisplatin mice. Values represent mean paw threshold ± SE (n= 4-5 per group). * denotes significant attenuation of tactile allodynia compared to the vehicle group ($p<0.05$).

Experiment 2&3

The effects of CBD and THC in preventing cisplatin tactile allodynia are summarized in Figure 3a and b. In both studies, cisplatin lowered paw withdrawal thresholds on D12 from baseline measures taken on D1 in the cisplatin administration protocol. CBD nor THC, at any doses tested, affected the eVF measures. One-way ANOVAs on each data set failed to reveal significant main effects of either THC or CBD dose (ps = n.s.). No further analyses were conducted on these data.

Figure 3: Effects of CBD and THC Prevention on Tactile Allodynia



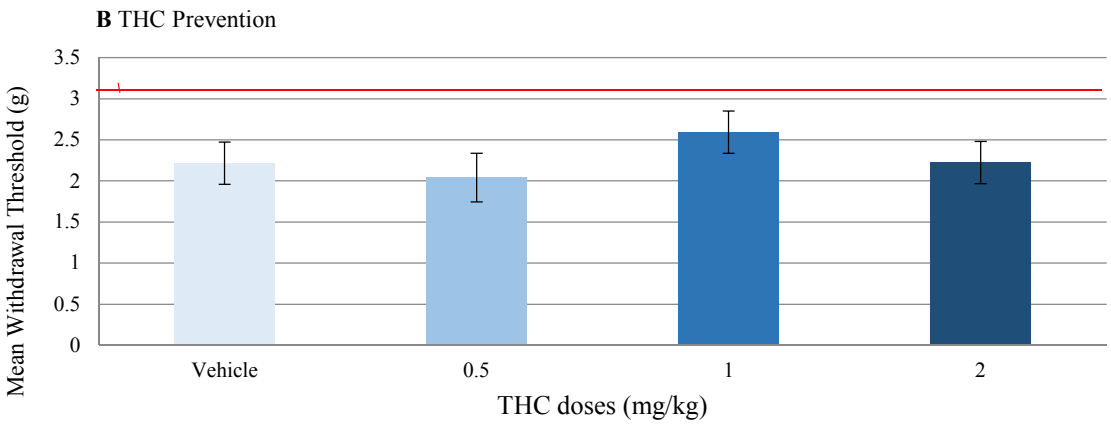


Fig 3. A) Effects of 6 dosings of saline and CBD administered prior to cisplatin across 13 days on tactile allodynia. Values represent mean paw withdrawal threshold ± SE (n=3-4 per group). B) Effects of 6 dosings of saline and THC administered prior to cisplatin across 13 days on tactile allodynia. Values represent mean paw withdrawal threshold ± SE (n=3-4 per group).

CHAPTER 5

DISCUSSION

The goals of this research were to examine whether the cannabinoid constituents THC or CBD can attenuate or prevent CIN. Experiment 1 was conducted to examine the effects of THC or CBD alone in attempts to attenuate CIN induced tactile allodynia. CIN was induced by administration of cisplatin 2.3 mg/kg/ml on alternating days for a total of nine cisplatin administrations. Tactile allodynia was quantified by applying microfilaments to the hind paws of mice and measuring the mean withdrawal threshold in grams. After the ninth cisplatin injection, mice receiving cisplatin had lower mean withdrawal thresholds than mice receiving vehicle. This tactile allodynic response is indicative of neuropathy. Neuropathy was confirmed and animals then were assigned to drug groups of vehicle, gabapentin 100mg/kg/ml, THC 2.0 mg/kg/ml, and CBD 2.0mg/kg/ml. Animals were administered their assigned drug 45 minutes prior to tactile measurements carried over 2 days. Experiments 2 and 3 were conducted to examine the effects of THC and CBD administered prior to cisplatin administration in attempts to prevent CIN induced tactile allodynia. Mice were pretreated with various doses of THC and CBD 30 minutes prior to cisplatin administration of 1.0mg/kg/ml and 2.3mg/kg/ml respectively for a total of 6 cisplatin administrations.

The development of neuropathy in all three experiments is consistent with previous research showing tactile allodynia can be induced under various cisplatin administration protocols and

doses. For example, protocols administering 2.3mg/kg/ml of cisplatin 6 times over 2 weeks as well as protocols administering 1 mg/kg/ml of cisplatin using a 5 day cycle over three weeks can produce cisplatin neuropathy (Park et al., 2012; Ta et al., 2009). Experiment 1 demonstrated that THC and CBD alone can produce an attenuation of cisplatin-induced tactile allodynia similar to that of gabapentin. This effect was evident where THC and CBD increased the mean withdrawal threshold comparable to gabapentin. This is consistent with literature that shows targeting the endocannabinoid system with synthetic cannabinoid compounds can attenuate allodynia associated with cisplatin. To our knowledge, this is the first attempt to study CBD in a CIN murine model and show a potential clinical benefit of this cannabinoid constituent in an oncology setting.

Unfortunately, Experiment 2 and 3 failed to prevent the onset of CIN with THC and CBD when administered prophylactically. Mice across all doses of THC and CBD showed a significant decrease in mean withdrawal threshold indicative of CIN. To our knowledge, this is the first attempt to use THC and CBD as a preventative for the onset of CIN. These findings suggest THC and CBD cannot slow down or prevent damage on nerve fibers produced by cisplatin.

Our findings suggest that CBD and THC alone may be beneficial in the treatment of CIN and a viable alternative to Sativex®. Collectively, the current studies fit well with the existing literature on the role of cannabinoid modulation of CIN. For example, Vera et al. demonstrated that cisplatin tactile allodynia in rats can be attenuated by administration of the CB selective and non-selective receptor agonists ACEA, JWH133 and WIN 55,212-2 (Vera at al., 2013). Cisplatin tactile allodynia can also be attenuated via through increased levels of the endocannabinoids anandamide and 2-arachidonylglycerol (2AG) via inhibition of fatty-acid amide hydrolase

(FAAH) and monoacylglycerol lipase (MGL), respectively (Guindon et al., 2012). These data, along with our findings suggest, that THC and CBD alone may prove clinically efficacious in treating CIN in oncology settings.

Limitations

Reliance of experimenter test administration and measurements are common limitations in behavioral research. The protocol of inducing CIN with cisplatin has shown to take as little as 5 cisplatin injections to achieve tactile allodynia, however our experiment needed additional cisplatin injections and a longer time frame. Difficulty in measuring tactile allodynia using an eVF as well as having multiple experimenters administering cisplatin could contribute to the additional cisplatin injections. An obvious limitation of these experiments is small sample sizes. Due to a rigorous protocol of measuring tactile responses with an eVF, time constraints limited the amount of animals used per study.

Future Research

Although the endocannabinoid system presents a rational target for the development of analgesics for oncology patients, natural product cannabinoids also play a significant role in cancer treatment (Pisnati et al., 2013). While a literature exists that targeting the endocannabinoid system inhibits tumor cell proliferation and progression and induces apoptosis and autophagy, there is also a literature that reports that endocannabinoid markers (receptors affinity, anandamide and 2AG) are upregulated in a number of highly malignant tumor types (see Pisanti et al., 2013 for review). Evidence suggests that these contradictory findings may reflect dose-dependent effects where macromolar concentrations of exogenous CBs have antitumor

properties while clinically-relevant micromolar doses have tumor promoting properties. A similar bi-phasic dose-effect is also observed with THC (Hart et al., 2004). For some cancers, THC has a dose-dependent effect on tumor cell pathogenesis where low concentrations increase and high concentrations decrease tumor cell proliferation (Alexander et al., 2009). In others, like breast cancer, THC increases tumor growth and metastasis while suppressing antitumor immune functions (McKallip et al., 2005). THC can produce dysphoria when administered alone, which further limits its usefulness in clinical settings (Alexander et al., 2009). The clinical shortcomings of THC stand in contrast to the potential benefits of the non-psychoactive cannabinoid, CBD. CBD has been shown to inhibit breast cancer growth and metastasis (Elbaz et al., 2015) and induce apoptosis in leukemia (McKallip et al., 2006). Along with the observation that CBD moderately affects tactile allodynia in a CIN model, CBD alone may be a viable alternative than THC in oncology settings.

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CONFERENCE PAPERS AND ABSTRACTS

Melissa J. Loria, Hannah M. Harris, Kevin Lewellyn, Jordan K. Zjawiony, Zulfiqar Ali, Ikhlas A. Khan, Kenneth J. Sufka. (April 2013). Evaluating preference-seeking and aversive qualities of salvia divinorum and mitragyna speciosa. 12th Annual Oxford International Conference on the Science of Botanicals.

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