



Cannabinoid mechanisms contribute to the therapeutic efficacy of the kratom alkaloid mitragynine against neuropathic, but not inflammatory pain

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ARTICLE INFO

Keywords:

Kratom
Mitragynine
Cannabinoids
Neuropathic pain

ABSTRACT

Aims: Mitragynine (MG) is an alkaloid found in *Mitragyna speciosa* (kratom), a plant used to self-treat symptoms of opioid withdrawal and pain. Kratom products are commonly used in combination with cannabis, with the self-treatment of pain being a primary motivator of use. Both cannabinoids and kratom alkaloids have been characterized to alleviate symptoms in preclinical models of neuropathic pain such as chemotherapy-induced peripheral neuropathy (CIPN). However, the potential involvement of cannabinoid mechanisms in MG's efficacy in a rodent model of CIPN have yet to be explored.

Main methods: Prevention of oxaliplatin-induced mechanical hypersensitivity and formalin-induced nociception were assessed following intraperitoneal administration of MG and CB1, CB2, or TRPV1 antagonists in wildtype and cannabinoid receptor knockout mice. The effects of oxaliplatin and MG exposure on the spinal cord endocannabinoid lipidome was assessed by HPLC-MS/MS.

Key findings: The efficacy of MG on oxaliplatin-induced mechanical hypersensitivity was partially attenuated upon genetic deletion of cannabinoid receptors, and completely blocked upon pharmacological inhibition of CB1, CB2, and TRPV1 channels. This cannabinoid involvement was found to be selective to a model of neuropathic pain, with minimal effects on MG-induced antinociception in a model of formalin-induced pain. Oxaliplatin was found to selectively disrupt the endocannabinoid lipidome in the spinal cord, which was prevented by repeated MG exposure.

Significance: Our findings suggest that cannabinoid mechanisms contribute to the therapeutic efficacy of the kratom alkaloid MG in a model of CIPN, which may result in increased therapeutic efficacy when co-administered with cannabinoids.

1. Introduction

Kratom (*Mitragyna speciosa*) is a plant native to Southeast Asia that has been used for centuries as a traditional medicine and herbal remedy. Kratom is consumed orally by chewing fresh or dried leaves, drinking crushed or boiled leaves in a tea, or by consuming encapsulated leaf powder [1–4]. In the United States, there are an estimated 10–16 million kratom users [3,5–9]. Kratom is comprised of over 40 unique alkaloid constituents, with mitragynine (MG) being the most prominent, accounting for roughly 66 % of the crude base alkaloid content and 6 % of

the plant's dried weight [10]. MG possesses a unique, mixed pharmacological profile combining opioid, α -adrenergic, and serotonergic receptor activity [11–17]. There is a wide range of self-proclaimed benefits of kratom use, ranging from mild stimulant effects to being used for pain relief and the mitigation of opioid withdrawal symptoms [18,19]. However, most of our understanding of the therapeutic benefits of kratom in humans is primarily anecdotal and based on anonymous self-report surveys. Interestingly, these surveys have revealed that prior or concurrent substance use is common among kratom users, particularly cannabis [5,7,20–22]. Kratom and cannabis products such as CBD

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<https://doi.org/10.1016/j.lfs.2023.121878>

Received 17 March 2023; Received in revised form 13 June 2023; Accepted 21 June 2023

Available online 29 June 2023

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are commonly sold together in major U.S. metropolitan areas, including 40.3 % of vape shops in 2021 [23]. The self-treatment of pain has been repeatedly listed as a primary motivator and benefit of kratom and cannabis use, particularly for those with underlying chronic neuropathic pain [20,22,24,25].

One of such neuropathic pain conditions is chemotherapy-induced peripheral neuropathy (CIPN), a debilitating and dose-limiting form of peripheral neuropathy that develops following chemotherapeutic exposure [26]. Current treatment options for CIPN include antidepressants, anticonvulsants, and opioids, which are limited in their efficacy and are associated with severe risk of adverse effects [27–31]. Therefore, there is an unmet need for novel pharmacological treatments for CIPN. Our previous studies demonstrate that MG prevents the development of mechanical hypersensitivity in rodent models of CIPN, which is mediated through μ -opioid and α -adrenergic receptor signaling [32,33]. Additionally, cannabinoids have been repeatedly demonstrated to improve CIPN symptomatology [34–38]. Cannabinoid mechanisms of MG have been previously explored, but not yet in the context of neuropathic pain [39,40].

To address the role of cannabinoid pharmacology in MG's analgesic effects, we used both genetic and pharmacological approaches to determine whether cannabinoid receptor signaling is required for MG-induced analgesia in models of neuropathic and inflammatory pain. Next, we explored potential functional consequences of MG on cannabinoid receptor signaling in vitro. Lastly, we aimed to characterize the potential impact of oxaliplatin and MG exposure on endocannabinoid lipidomics and metabolism. Overall, the findings reported in this manuscript support the involvement of cannabinoid signaling in the anti-allodynic efficacy of MG in the context of CIPN. Given that kratom and cannabis products are commonly used in combination and are often used for the self-treatment of pain, identifying a mechanistic overlap in their abilities to alleviate symptoms of CIPN may uncover previously unrecognized benefits of simultaneous use for treating chronic neuropathic pain.

2. Materials and methods

2.1. Animals

Both male and female wildtype C57BL/6 mice (8–10 weeks) were purchased from The Jackson Laboratory (Bar Harbor, ME). $Cnr1^{-/-}/Cnr2^{-/-}$ double knockout mice on a C57BL/6 background were generated and maintained inhouse at Temple University. All animals were group-housed under standard conditions on a 12-h light/dark cycle. Mice were allowed to acclimate to the animal facilities for 5 days prior to the beginning of experimentation. Food and water were available ad libitum throughout all experiments. Animals were randomly assigned to treatment groups for all experiments. All animal use procedures were conducted in accordance with the National Research Council and the National Academic Press publication for the Care and Use of Laboratory Animals (adopted for use by the National Institutes of Health). All animal experimental procedures complied with the guidelines of the Temple University Institutional Animal Care and Use Committee (IACUC).

2.2. Drugs and chemicals

Oxaliplatin was obtained from Temple University Hospital Pharmacy (Philadelphia, PA) as a 6 mg/ml concentration stock solution and diluted in sterile water. Mitragynine (MG) was purchased from Cayman Chemical Company (Ann Arbor, MI) with molecular weight and purity (> 97.3 %) secondarily confirmed by Dr. Allen Reitz (Fox Chase Chemical Diversity Center, Doylestown, PA). MG was dissolved in a vehicle of 20 % Tween 80 and 80 % sterile water. SR141716 and SR144528 were purchased from Cayman Chemical Company and dissolved in a vehicle of 1:1:18 ethyl alcohol:cremophor:saline (v/v).

Capsazepine was purchased from Cayman Chemical Company and dissolved in a vehicle of 2 % dimethyl sulfoxide (DMSO), 10 % Tween 80, and 88 % saline. For in vitro studies, the non-selective cannabinoid receptor agonist CP55,940 was obtained from Cayman Chemical Company and dissolved in DMSO. Formalin solution was purchased from Sigma Aldrich (St. Louis, MO) and diluted using sterile saline. For in vivo studies, all drugs were administered intraperitoneally (i.p.) at a volume of 0.1 ml/10 g body weight.

2.3. Chemotherapy-induced peripheral neuropathy (CIPN)

To measure the effects of oxaliplatin and MG on mechanical hypersensitivity, mechanical allodynia was measured using von Frey monofilaments (0.07–2.0 g) applied to the plantar surface of the right hind paw, with each application being held in a c-shape for 6 s. A modified up-down method was used as previously described [41]. The smallest bending force to elicit a nocifensive response was indicative of an animal's mechanical threshold. Mice were placed in individual Plexiglas holder compartments (Bioseb, Pinellas Park, FL) placed on top a wire grid floor suspended 30 cm above the bench top and acclimated to the apparatus environment for 30 min before testing. Baseline mechanical sensitivity was measured for each animal just prior to the start of drug administration on experimental day 0. Following baseline measurements, mice received a single injection of vehicle or oxaliplatin (6 mg/kg) to induce mechanical hypersensitivity. Mechanical allodynia was additionally measured on experimental days 2, 5 and 7. MG (20 mg/kg) or vehicle was then administered once daily from experimental days 1–6. For mechanistic studies, SR141716 (1, 3, 10 mg/kg), SR144528 (1, 3, 10 mg/kg) or capsazepine (10 mg/kg) was administered prior to MG (Supplemental Fig. 1). The SR141716 and SR144528 dose range was selected based on previous studies in taxane and cisplatin models of CIPN [42–46]. On days of simultaneous behavioral assessment and MG treatment, MG was administered 15 min following behavioral testing to circumvent potential acute analgesic effects of MG exposure. Both male and female $Cnr1^{-/-}/Cnr2^{-/-}$ mice were used, whereas WT mice were male only. Antagonists were tested in male WT animals only.

2.4. Formalin test

To study whether MG would display antinociception against inflammatory pain, the formalin-induced pain model was implemented as previously described [47,48]. Following acclimation to the experimental environment, mice were injected with either vehicle or MG (20 mg/kg). For antagonist studies, capsazepine (10 mg/kg), SR141716 (3–10 mg/kg), SR144528 (3–10 mg/kg) or vehicle were injected 15 min prior to MG exposure. Formalin (20 μ l, 5 %) was injected subcutaneously into the plantar surface of the right hind paw 40 min after MG exposure (Supplemental Fig. 2). Mice were individually placed into glass observation boxes immediately following intraplantar formalin injection and were observed for licking response of the injected paw. Licking responses were observed first the 10 min (Phase I) and then 20–35 min (Phase II) post-formalin injection. Both male and female $Cnr1^{-/-}/Cnr2^{-/-}$ and WT mice were used. Antagonists were tested in WT animals only.

2.5. Real-time qRT-PCR analysis

Lumbar spinal cords were dissected from mice treated with vehicle, oxaliplatin, oxaliplatin + MG (20 mg/kg), or vehicle + MG (20 mg/kg), and total RNA was extracted using a Quick-RNA™ MiniPrep kit (Zymo Research, Irvine, CA) according to manufacturer's instructions. All RNA samples had A260/A280 ratios of 1.8 to 2.0. Purified RNA was treated with DNase I and eluted with DNase/RNase-free water. Reverse transcription was performed using an RT² First Strand cDNA kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Quantitative real-time PCR assays were performed using Taq Man Gene

Expression Assays (Thermo Scientific, Waltham, MA) to quantify mRNA levels of cannabinoid receptor 1 (Cnr1, ref. Mm00432621_s1), cannabinoid receptor 2 (Cnr2, ref. Mm00438286_m1), diacylglycerol lipase alpha (Dagl- α , ref. Mm00813830_m1), fatty acid amide hydrolase (Faah, ref. Mm00515684_m1), monoacylglycerol lipase (Mgl1, ref. Mm00449274_m1), and N-acyl phosphatidylethanolamine phospholipase D (Nape-pld, ref. Mm00724596_m1), using the 18S ribosomal RNA probe (Rn18s, ref. Mm03928990_g1) as an internal control. The PCR assay was performed using a QuantStudio™ 3 Real-Time PCR System (Thermo Scientific), with the threshold cycle (Ct) calculated by the instrument's software. Each reaction was run in triplicate and contained 50–100 ng of RNA in a final reaction volume of 20 μ l. Expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method.

2.6. cAMP Response Element (CRE) assays

HEK293 cells were transiently transfected with CB1 or CB2 and CRE-luc vector reporter plasmids using Lipofectamine 2000 as described by the manufacturer (Invitrogen, Waltham, MA). Transfected HEK293 cells were seeded (60,000 cells/well) in 96-well plates. Five hours later, the medium was changed to a 1 % FBS/DMEM mixture. Cells were incubated overnight. The next day, cells were treated with ligands (CP55,940, MG, SR141716, or SR144528) for 5 h in serum-free DMEM medium at 37 °C. After treatment, cells were lysed with 1 \times lysis buffer for 10 min at room temperature. Plates were read to record bioluminescence immediately following 40 μ l Luciferin (≥ 250 μ M) per well. Luminescence was measured in an Envision 2104 multilabel reader (PerkinElmer, Waltham, MA). Luminescence values are given as relative light units, which were used to make concentration-effect curves and analyzed by nonlinear regression techniques.

2.7. HPLC-MS/MS

For lipid extraction, tissue was processed as previously described for CNS analysis [49]. On the day of processing spinal cord samples, ~50 vol of methanol were added to each sample in addition to 5 μ l of 1 μ M d8AEA. Samples were incubated in the dark on ice for 2 h, then homogenized via sonication, and centrifuged at 19,000G, 20 °C, for 20 min. Supernatants were added to HPLC-grade water to make a 75 % organic solution. Lipids were partially purified from this mixture using C-18 solid phase extraction columns (Agilent, Santa Clara, CA), eluting with 65 %, 75 %, and 100 % methanol (1.5 ml).

2.8. Statistical analysis

All statistical analyses were conducted using GraphPad Prism 9 software. One-way analysis of variance (ANOVA) was performed when comparisons were made across more than two groups. Two-way ANOVA was used to test differences between two or more groups across different time points or genotypes. Dunnett's or Tukey's post-hoc analyses were used to detect individual differences between groups in the instance of significance by ANOVA. Fisher's least significant difference (LSD) test was used to detect individual differences between groups in molecular, non-behavioral studies. All data are represented as mean \pm SEM.

For HPLC-MS/MS experiments, 20 μ l of each sample was assessed for related lipid species up to 6 lipids in one mass spectrometric analysis. Levels of individual lipids were determined based on concentration curves of known standards. Final moles/g calculations were adjusted based on the average percent recovery of the internal standard and amount of starting material. Outliers in individual endolipids beyond two standard deviations of the mean were excluded in final analyses. Data were analyzed with one-way ANOVAs with Fisher's Least Significant Differences post-hoc (vehicle compared to drug treatment; oxaliplatin compared to drug treatment). Significance was set $P \leq 0.05$, and trending significance as $0.05 < P \leq 0.10$. Analyzed data are represented in tabular format (heatmaps) and illustrate both the direction and fold

change as well as significance. To determine the fold change and therefore the number of arrows to assign each significant difference, the mean level of a particular lipid was divided by that same lipid's mean level with a different treatment (either vehicle or oxaliplatin). This analytical procedure was previously described in detail [50].

3. Results

3.1. The anti-allodynic, but not the antinociceptive effects of MG are disrupted in Cnr1^{-/-} / Cnr2^{-/-} mice

To characterize the potential role of cannabinoid receptor signaling in the anti-allodynic and antinociceptive effects of MG, we first tested MG in CIPN and formalin pain models in both wildtype and Cnr1^{-/-} / Cnr2^{-/-} animals. For mechanical allodynia, two-way ANOVA revealed a significant main effect of time [F (2.227, 56.41) = 86.82, $P < 0.0001$], treatment [F (3, 27) = 5.951, $P < 0.01$], and interaction [F (9, 76) = 4.078, $P < 0.001$] (Fig. 1A). Tukey's multiple comparisons test reported that mean mechanical threshold values of WT oxaliplatin + MG animals were significantly greater than WT oxaliplatin alone across days 2–7, indicative of an anti-allodynic effect. The mean mechanical threshold of Cnr1^{-/-} / Cnr2^{-/-} oxaliplatin + MG was significantly higher than Cnr1^{-/-} / Cnr2^{-/-} oxaliplatin on day 2 only, suggesting that the anti-allodynic effect of MG was lost on days 5 and 7 in Cnr1^{-/-} / Cnr2^{-/-} animals. When comparing oxaliplatin + MG groups across genotypes, no significant differences were detected.

In the formalin test, two-way ANOVA for acute pain (Phase I) revealed a significant main effect for treatment [F (1, 22) = 15.67, $P < 0.001$] and genotype [F (1, 22) = 11.57, $P < 0.01$] (Fig. 1B). No significant main effect was found for interaction [F (1, 22) = 1.134, $P = 0.299$]. Tukey's multiple comparisons test reported that mean licking time was significantly reduced in WT MG-treated mice compared to WT controls ($P < 0.05$). Mean licking time was also significantly reduced in Cnr1^{-/-} / Cnr2^{-/-} controls compared to WT controls, suggesting diminished inflammatory nociception in Cnr1^{-/-} / Cnr2^{-/-} animals ($P < 0.05$). However, MG-treated WT animals were not significantly different than Cnr1^{-/-} / Cnr2^{-/-} MG-treated animals. Two-way ANOVA analysis for persistent pain (Phase II) revealed a significant main effect for treatment [F (1,21) = 25.65, $P < 0.001$] and genotype [F (1, 21) = 30.66, $P < 0.001$] (Fig. 1C). No significant main effect for interaction was detected [F (1, 21) = 1.465, $P = 0.239$]. Tukey's multiple comparisons test revealed that MG significantly reduced mean licking time in both WT and Cnr1^{-/-} / Cnr2^{-/-} compared to vehicle ($P < 0.01$ and $P < 0.05$, respectively). Mean licking time was significantly lower in MG-treated Cnr1^{-/-} / Cnr2^{-/-} mice compared to WT animals treated with MG ($P < 0.05$). However, mean licking times were also significantly lower in vehicle-treated Cnr1^{-/-} / Cnr2^{-/-} mice compared to WT vehicle animals, again suggesting diminished inflammatory pain response in Cnr1^{-/-} / Cnr2^{-/-} animals ($P < 0.001$).

3.2. Selective CB1 antagonism blocks the anti-allodynic, but not the antinociceptive effects of MG

To improve our understanding of the contributions of cannabinoid signaling in the antinociceptive and anti-allodynic effects of MG, we next explored the contributions of each cannabinoid receptor independently through pharmacological antagonism. First, the impact of CB1 receptor signaling to the anti-allodynic effect of a fixed dose of MG (20 mg/kg) in WT animals treated with oxaliplatin was evaluated by pre-treatment with a dose range of the CB1-selective antagonist SR141716 (1, 3, 10 mg/kg) (Fig. 2A). In the CIPN assay, two-way ANOVA revealed a significant main effect for time [F (2.826, 133.3) = 59.84, $P < 0.0001$], treatment [F (6, 49) = 13.23, $P < 0.0001$], and interaction [F (18, 142) = 3.958, $P < 0.0001$]. Oxaliplatin alone induced mechanical hypersensitivity starting on day two that persisted throughout the duration of the study. Dunnett's multiple comparisons test revealed that the mean

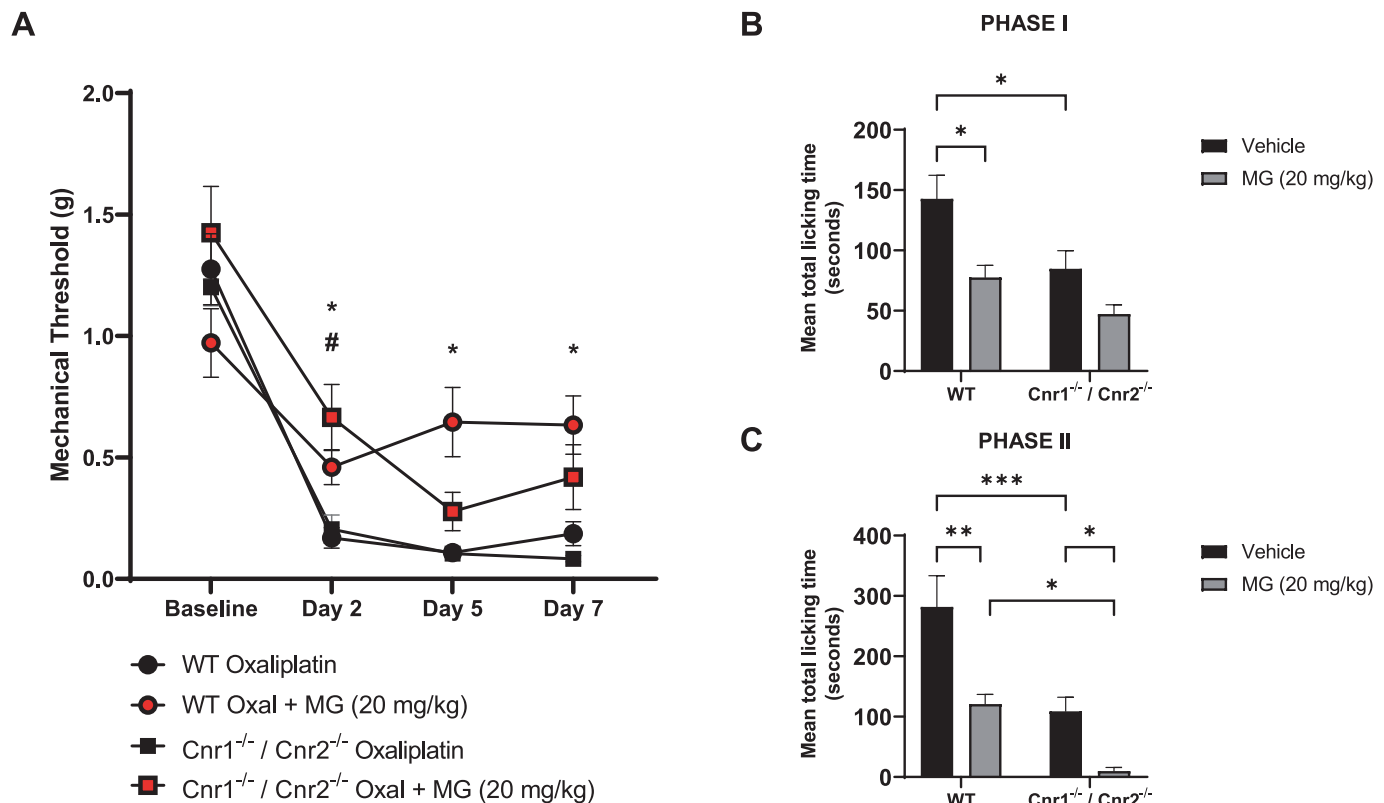


Fig. 1. Genetic deletion of cannabinoid receptors contributes to the anti-allodynic, but not antinociceptive properties of MG. (A) The development of oxalipatin-induced mechanical hypersensitivity was prevented in wildtype mice treated with MG (male, $n = 8$), but not in $Cnr1^{-/-} / Cnr2^{-/-}$ mice treated with MG (mixed sex, $n = 8$). $*P < 0.05$ WT oxal + MG compared to WT controls, $\#P < 0.05$ $Cnr1^{-/-} / Cnr2^{-/-}$ oxal + MG compared to $Cnr1^{-/-} / Cnr2^{-/-}$ controls. (B) MG significantly reduced paw licking time in WT mice compared to WT controls (mixed sex, $n = 8$) in Phase I of the formalin test. $Cnr1^{-/-} / Cnr2^{-/-}$ controls (mixed sex, $n = 8$) exhibited significantly lower licking times compared to WT controls. MG-treated $Cnr1^{-/-} / Cnr2^{-/-}$ mice (mixed sex, $n = 8$) did not differ from $Cnr1^{-/-} / Cnr2^{-/-}$ controls or MG WT. $*P < 0.05$. (C) MG significantly reduced paw licking time in WT mice compared to WT controls in Phase II of the formalin test. $Cnr1^{-/-} / Cnr2^{-/-}$ controls exhibited significantly lower licking times compared to WT controls. MG-treated $Cnr1^{-/-} / Cnr2^{-/-}$ licking times were significantly lower than WT MG and $Cnr1^{-/-} / Cnr2^{-/-}$ controls. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. All data are represented as mean \pm SEM.

mechanical thresholds of oxalipatin + MG animals were significantly greater than oxalipatin alone across days 2–7, indicative of an anti-allodynic effect. Moreover, the mean mechanical thresholds of oxalipatin + MG animals were not significantly different than vehicle animals across all days of the experiment. Mean mechanical thresholds of animals pretreated with 10 mg/kg, but not 3 mg/kg or 1 mg/kg of SR141716 prior to MG were significantly lower than MG alone across days 2–7, suggesting that high doses of SR141716 are sufficient to block the anti-allodynic effect of MG.

In the formalin test, only 3 mg/kg and 10 mg/kg doses of SR141716 were tested based on the observation that 1 mg/kg did not elicit any effect on MG in the CIPN assay. In Phase I, two-way ANOVA revealed a significant main effect of MG treatment [$F(1, 37) = 5.625$, $P < 0.05$] but not for SR141716 pretreatment [$F(2, 37) = 0.3672$, $P = 0.695$] (Fig. 2B). A significant main effect for interaction was also revealed [$F(2, 37) = 4.919$, $P < 0.05$]. Tukey's multiple comparisons test revealed that the mean licking times of mice treated with MG alone were significantly lower than vehicle-treated mice, indicative of acute antinociception ($P < 0.05$). Mice treated with 3 mg/kg of SR141716 also exhibited significantly lower mean licking times than vehicle, suggesting that this dose is insufficient to block MG-induced antinociception. SR141716 (10 mg/kg) + MG animals were neither significantly different from those treated with vehicle or MG alone, suggesting partial blockade of MG-induced antinociception. Neither dose of SR141716 tested alone was significantly different than control. In phase II, a significant main effect was found for MG treatment [$F(1, 36) = 60.94$, $P < 0.0001$], but not SR141716 pretreatment [$F(2, 36) = 1.625$, $P = 0.211$] or interaction [$F(2, 36) = 0.1328$, $P = 0.876$] (Fig. 2C). Mean licking times of mice

treated with MG alone were significantly lower than vehicle, suggesting an antinociceptive effect of MG against persistent inflammatory pain. Both SR141716 + MG treatment groups exhibited mean licking times that were also significantly lower than vehicle, suggesting that the doses of SR141716 tested were not sufficient to inhibit the antinociceptive effect of MG in this phase.

3.3. Selective CB2 antagonism blocks the anti-allodynic, but not the antinociceptive effects of MG

Like that for CB1 receptor signaling, we also aimed to characterize the specific contributions of CB2 receptor signaling in the anti-allodynic and antinociceptive effects of MG. To assess the effect of CB2 receptor signaling on MG (20 mg/kg) against oxalipatin-induced mechanical allodynia in WT mice, mice were pretreated with a dose range of the CB2-selective inverse agonist SR144528 (1, 3, 10 mg/kg) (Fig. 3A). In the CIPN model, two-way ANOVA revealed a significant main effect for time [$F(2.575, 116.7) = 82.68$, $P < 0.0001$], treatment [$F(6, 47) = 27.72$, $P < 0.0001$], and interaction [$F(18, 136) = 4.485$, $P < 0.0001$]. Oxalipatin alone induced mechanical hypersensitivity starting on day two that persisted throughout the duration of the study. Dunnett's multiple comparisons test revealed that mean mechanical thresholds of oxalipatin + MG mice were significantly higher than oxalipatin alone across days 2–7. The mean mechanical thresholds of oxalipatin + MG mice were not significantly different from vehicle across all days of the experiment. Both 3 mg/kg and 10 mg/kg, but not 1 mg/kg of SR144528 were sufficient to block the anti-allodynic effect of MG, and this significant effect persisted across days 2–7.

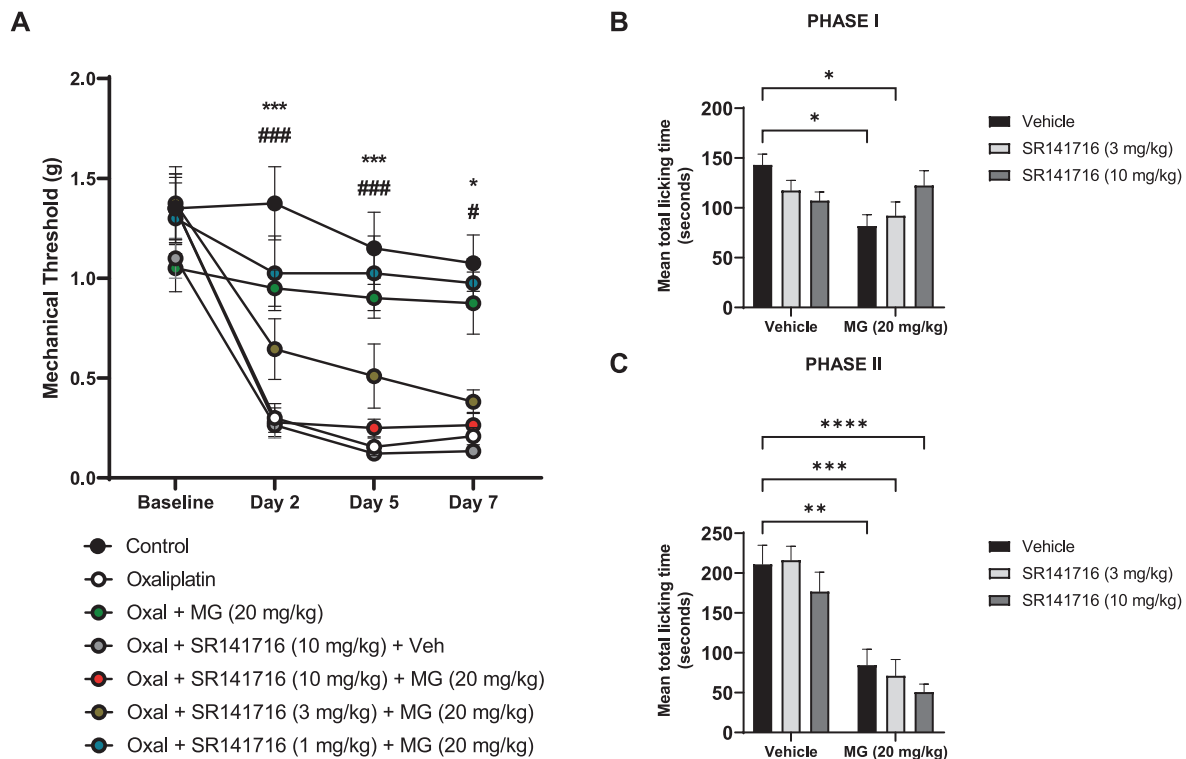


Fig. 2. Pharmacological inhibition of CB1 disrupts the anti-allodynic, but not the antinociceptive properties of MG. (A) High doses of SR141716 pretreatment inhibited the ability of MG to prevent oxaliplatin-induced mechanical hypersensitivity. Mechanical thresholds of oxal + MG mice were significantly higher than oxaliplatin alone, but not significantly different from controls across days 2–7. SR141716 (10 mg/kg) pretreatment produced mechanical thresholds significantly lower than oxal + MG across days 2–7. * $P < 0.05$, *** $P < 0.001$ oxal + MG compared to oxaliplatin alone. # $P < 0.05$, ### $P < 0.001$ oxal + MG compared to oxal + SR141716 (10 mg/kg) + MG. All groups male, $n = 8$. (B) SR141716 pretreatment did not inhibit the antinociceptive properties of MG in Phase I (B) or Phase II (C) of the formalin test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. All groups mixed sex, $n = 7$ –8. All data are represented as mean \pm SEM.

Based on the observation that 3 and 10 mg/kg of SR14428 were sufficient to block the anti-allodynic effect of MG, these two doses were subsequently tested against MG in the formalin test. In phase I, two-way ANOVA revealed a significant main effect for SR144528 pretreatment [$F(2, 47) = 5.03$, $P < 0.05$], but not for MG treatment [$F(1, 47) = 1.585$, $P = 0.214$] or interaction [$F(2, 47) = 0.549$, $P = 0.581$] (Fig. 3B). Here, post hoc analysis did not indicate a significant difference in mean licking time between vehicle controls and MG alone. However, mean licking times were significantly lower in MG-treated animals pretreated with SR144528 compared to vehicle alone ($P < 0.05$). Interestingly, treatment with 10 mg/kg of SR144528 alone was sufficient to significantly decrease mean licking time compared to vehicle controls ($P < 0.05$). In phase II, two-way ANOVA revealed a significant main effect for MG treatment [$F(1, 46) = 29.78$, $P < 0.0001$], but not for SR14428 pretreatment [$F(2, 46) = 0.011$, $P = 0.989$] or interaction [$F(2, 46) = 0.264$, $P = 0.769$] (Fig. 3C). Post hoc analysis revealed that the mean licking time of MG-treated mice were significantly lower than vehicle controls, indicative of an antinociceptive effect ($P < 0.01$). Mice pretreated with 3 mg/kg or 10 mg/kg of SR144528 also displayed significantly lower mean licking times compared to vehicle controls, suggestive that SR144528 is insufficient to inhibit the antinociceptive effect of MG at these doses tested ($P < 0.01$ and 0.05 , respectively). Contrary to SR141716, neither dose of SR144528 alone was sufficient to significantly reduce mean licking times.

3.4. MG does not modulate cannabinoid receptor second messenger signaling in vitro

Given that MG exhibited therapeutic effects in a CIPN model that was achieved by cannabinoid receptor activation, we next sought to investigate potential functional consequences of MG on cannabinoid receptor

signaling in vitro. Specifically, we measured the effects of MG on both CB1- and CB2-dependent cyclic AMP (cAMP)/PKA signaling (Fig. 4). The cAMP response element (CRE) response was suppressed by CP55,940 in both CB1- and CB2-transfected cells, corresponding to its potent, non-selective agonist properties at both CB1 and CB2 receptors. Treatment with SR141716 and SR144528 increased the CRE response in CB1- and CB2-transfected cells, respectively. However, treatment with MG did not produce a significant change in CRE response in either CB1- or CB2- transfected cells, suggesting that MG does not modulate cAMP signaling of cannabinoid receptors in vitro.

3.5. TRPV1 antagonism blocks the anti-allodynic, but not the antinociceptive effects of MG

Based on our findings that MG does not affect second messenger signaling systems of CB1 or CB2 receptors in vitro, we next aimed to explore the potential effects of MG at non-GPCR targets of endogenous and exogenous cannabinoid ligands. The transient receptor potential cation channel subfamily V member 1 (TRPV1) was identified as a target of interest based on supporting evidence of its role in cannabinoid signaling, the pathogenesis of CIPN, and the application of capsaicin patches for the treatment of neuropathic pain conditions [51,52]. To assess the effects of TRPV1 signaling on the anti-allodynic and antinociceptive effects of MG, mice were pretreated with the synthetic TRPV1 antagonist capsazepine (10 mg/kg). For mechanical allodynia, two-way ANOVA revealed a significant main effect for time [$F(2.824, 96.02) = 54.62$, $P < 0.0001$], treatment [$F(4, 35) = 25.23$, $P < 0.0001$], and interaction [$F(12, 102) = 6.172$, $P < 0.0001$] (Fig. 5A). Post-hoc analysis revealed that the mean mechanical thresholds of oxaliplatin + MG mice were significantly higher than oxaliplatin alone across days 2–7, and that oxaliplatin + MG was not significantly different from

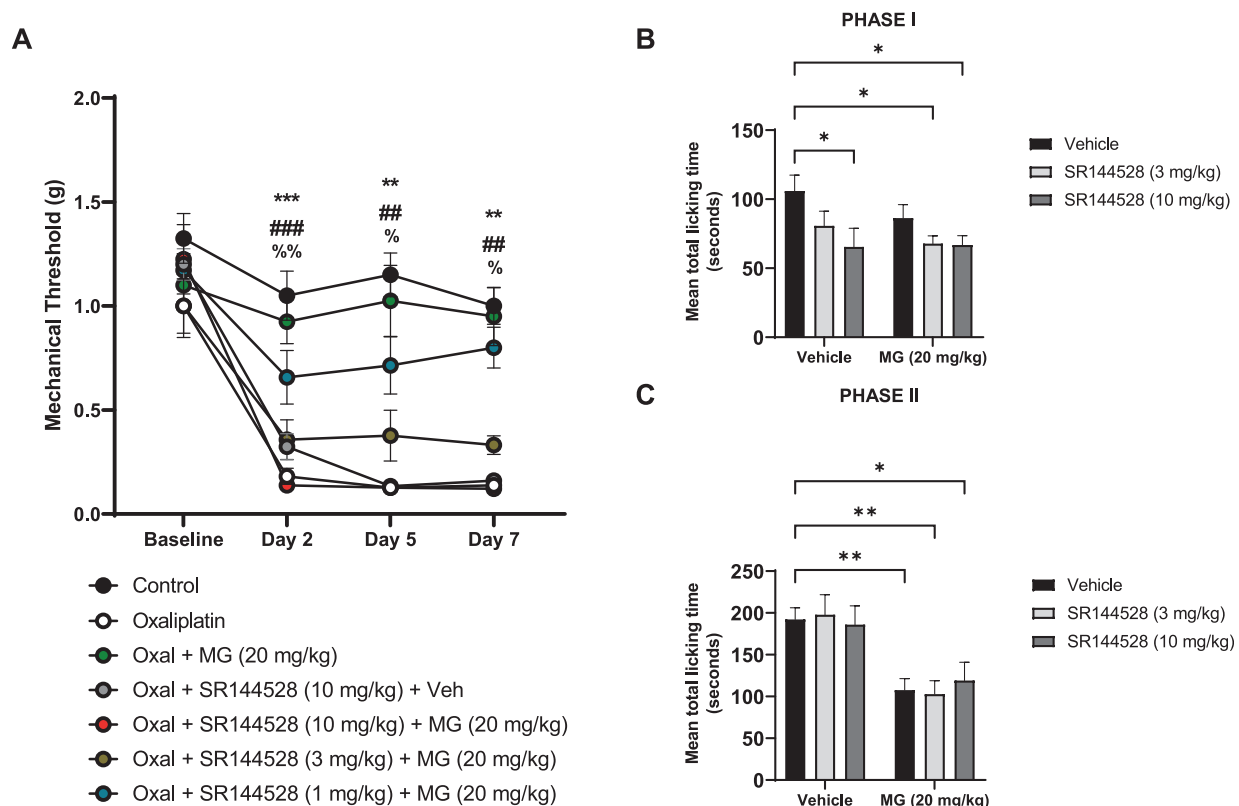


Fig. 3. Pharmacological inhibition of CB2 disrupts the anti-allodynic, but not the antinociceptive properties of MG. (A) SR144528 pretreatment dose dependently inhibited the ability of MG to prevent oxaliplatin-induced mechanical hypersensitivity. Mechanical thresholds of oxal + MG mice were significantly higher than oxaliplatin alone, but not significantly different from controls across days 2–7. SR144528 pretreatment at 3 mg/kg and 10 mg/kg significantly inhibited the anti-allodynic effects of MG across days 2–7. $^{**}P < 0.01$, $^{***}P < 0.001$ oxal + MG compared to oxal alone. $^{##}P < 0.01$, $^{###}P < 0.001$ oxal + MG compared to oxal + SR144528 (10 mg/kg) + MG. $^{%%}P < 0.05$, $^{%%%}P < 0.01$ oxal + MG compared to oxal + SR144528 (3 mg/kg) + MG. All groups male, $n = 8$. (B) High doses of SR144528 alone and in combination with MG resulted in significantly lower licking times than vehicle controls in Phase I of the formalin test. Pretreatment with SR144528 (3 mg/kg) prior to MG also produced licking times lower than controls. MG alone was not significantly different from vehicle controls. $^{*}P < 0.05$. All groups mixed sex, $n = 8–11$. (C) SR144528 pretreatment was insufficient to block the antinociceptive effects of MG in Phase II of the formalin test. Mean licking times of mice treated with MG alone or in combination with SR144528 produced were significantly lower than vehicle controls. $^{*}P < 0.05$, $^{**}P < 0.01$. All data are represented as mean \pm SEM.

control, indicative of an anti-allodynic effect. Mice pretreated with capsaizine prior to MG displayed significantly lower mean mechanical threshold values than those treated with oxaliplatin + MG on days 5 and 7, suggesting that TRPV1 antagonism blocked the anti-allodynic effect of MG.

In phase I of the formalin test, no significant main effects were detected by two-way ANOVA for MG treatment [$F(1, 27) = 2.309$, $P = 0.14$], capsaizine pretreatment [$F(1, 27) = 1.571$, $P = 0.221$], or interaction [$F(1, 27) = 0.388$, $P = 0.534$] (Fig. 5B). In phase II, a significant main effect was found for MG treatment [$F(1, 27) = 9.501$, $P < 0.01$], but not capsaizine pretreatment [$F(1, 27) = 0.298$, $P = 0.589$] or interaction [$F(1, 27) = 2.276$, $P = 0.143$] (Fig. 5C). Tukey's multiple comparisons test revealed that the mean licking time was significantly lower in MG-treated animals compared to vehicle controls ($P < 0.05$). A trending, but statistically nonsignificant effect was found between capsaizine + MG-treated animals compared to MG alone ($P = 0.0655$).

3.6. Oxaliplatin does not disrupt the mRNA expression of endocannabinoid biosynthetic or hydrolytic enzymes or cannabinoid receptors in the spinal cord

To further improve our understanding of the molecular interactions between oxaliplatin and MG within the lumbar spinal cord, we sought to characterize the potential effects oxaliplatin and MG may have on the mRNA expression of key enzymes involved in the biosynthesis and

hydrolysis of the endocannabinoids 2-AG and AEA, in addition to CB1 and CB2 receptors. Mice were treated with a single injection of oxaliplatin or vehicle, followed by six consecutive days of MG or vehicle. Lumbar spinal cords were harvested and mRNA expression of Nape-pld, Faah, Dagl- α , MglI, Cnr1, and Cnr2 was assessed (Fig. 6). A main effect of treatment was detected by one-way ANOVA for Nape-pld [$F(3, 28) = 6.234$, $P < 0.01$], Dagl- α [$F(3, 30) = 7.713$, $P < 0.001$], MglI [$F(3, 28) = 3.124$, $P < 0.05$], and Cnr1 [$F(3, 24) = 3.694$, $P < 0.05$]. Fold change in expression of Nape-pld, Dagl- α , and Cnr1 were significantly lower in mice treated with MG alone compared to those treated with oxaliplatin + MG. Moreso, mRNA expression of Nape-pld, Dagl- α , and MglI were significantly lower in animals treated with MG alone compared to oxaliplatin alone. Expression of Dagl- α was significantly lower in mice treated with MG alone compared to controls. Nape-pld was the only target in which fold change in expression was significantly different between control and oxaliplatin animals. Importantly, no significant differences were detected between animals treated with oxaliplatin alone or oxaliplatin + MG.

3.7. MG restores oxaliplatin-induced selective disruption of the spinal cord endocannabinoid lipidome

Based on the observation that cannabinoid receptor and TRPV1 activation only contributed to the therapeutic efficacy of MG in a model of CIPN, we next aimed to further characterize the underlying

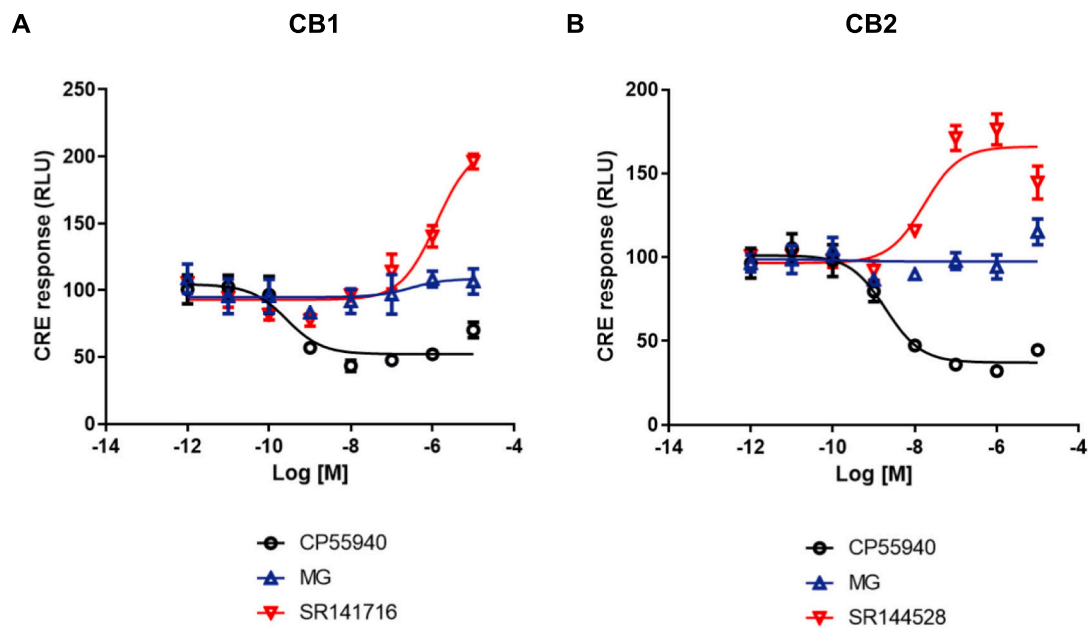


Fig. 4. Effects of MG on cannabinoid-mediated inhibition of cAMP signaling in vitro. (A) Dose response effects of CP55,940, MG, and SR141716 on CRE response in HEK-293-CB1R cells. CP55,940 dose-dependently decreased CRE response, whereas SR141716 dose-dependently increased CRE response. (B) Dose response effects of CP55,940, MG, and SR144528 on CRE response in HEK-293-CB2R cells. CP55,940 dose-dependently decreased CRE response, whereas SR144528 dose-dependently increased CRE response. MG produced no effect in either condition. All data are represented as mean \pm SEM.

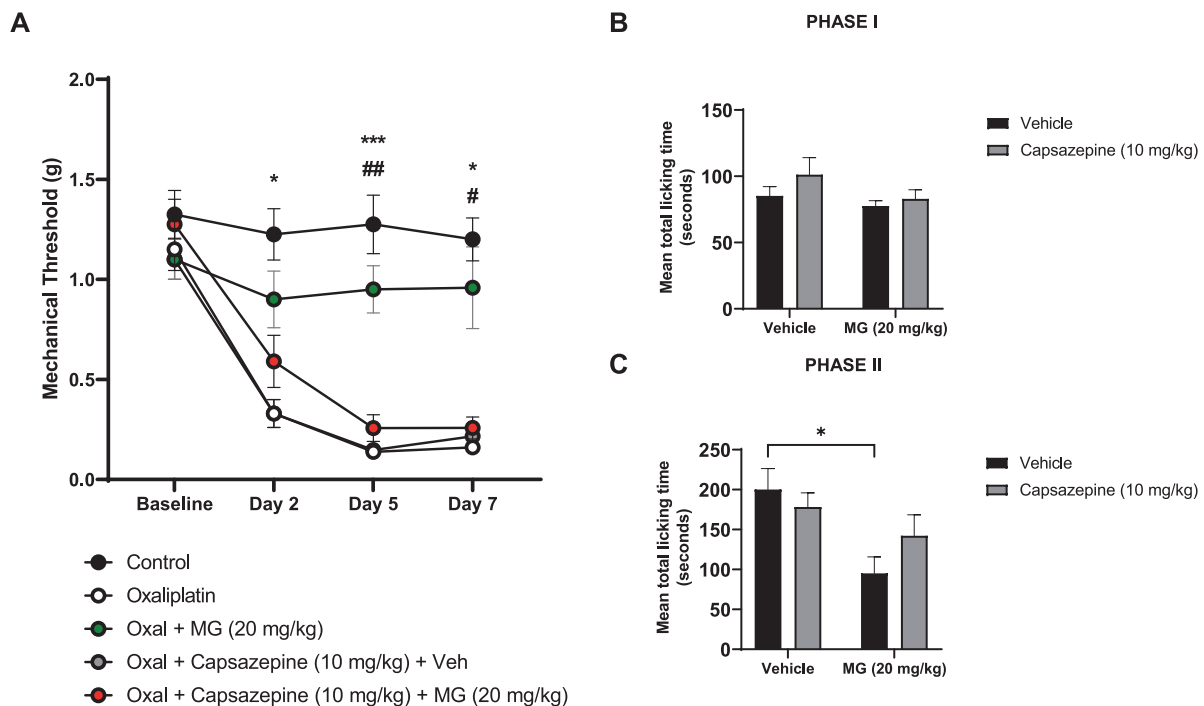


Fig. 5. Pharmacological inhibition of TRPV1 disrupts the anti-allodynic, but not the antinociceptive properties of MG. (A) Pretreatment with capsazepine (10 mg/kg) prior to MG inhibited the anti-allodynic effects of MG on days 5 and 7. Mechanical thresholds of oxal + MG mice were significantly greater than oxaliplatin alone, but not significantly different from controls across days 2–7. * $P < 0.05$, *** $P < 0.001$ oxal + MG compared to oxaliplatin alone. # $P < 0.05$, ## $P < 0.01$ oxal + MG compared to oxal + capsazepine (10 mg/kg) + MG. All groups male, $n = 8$. (B) Capsazepine pretreatment prior to MG did not significantly change mean licking times compared to MG alone in Phase I of the formalin test. MG alone was not significantly different from vehicle controls. All groups mixed sex, $n = 7$ –8. (C) Capsazepine pretreatment was insufficient to inhibit the antinociceptive effect of MG in Phase II of the formalin test. Mean licking times in MG-treated mice were significantly lower than vehicle controls. Mice treated with both capsazepine and MG did not significantly differ from MG alone or vehicle controls. * $P < 0.05$. All data are represented as mean \pm SEM.

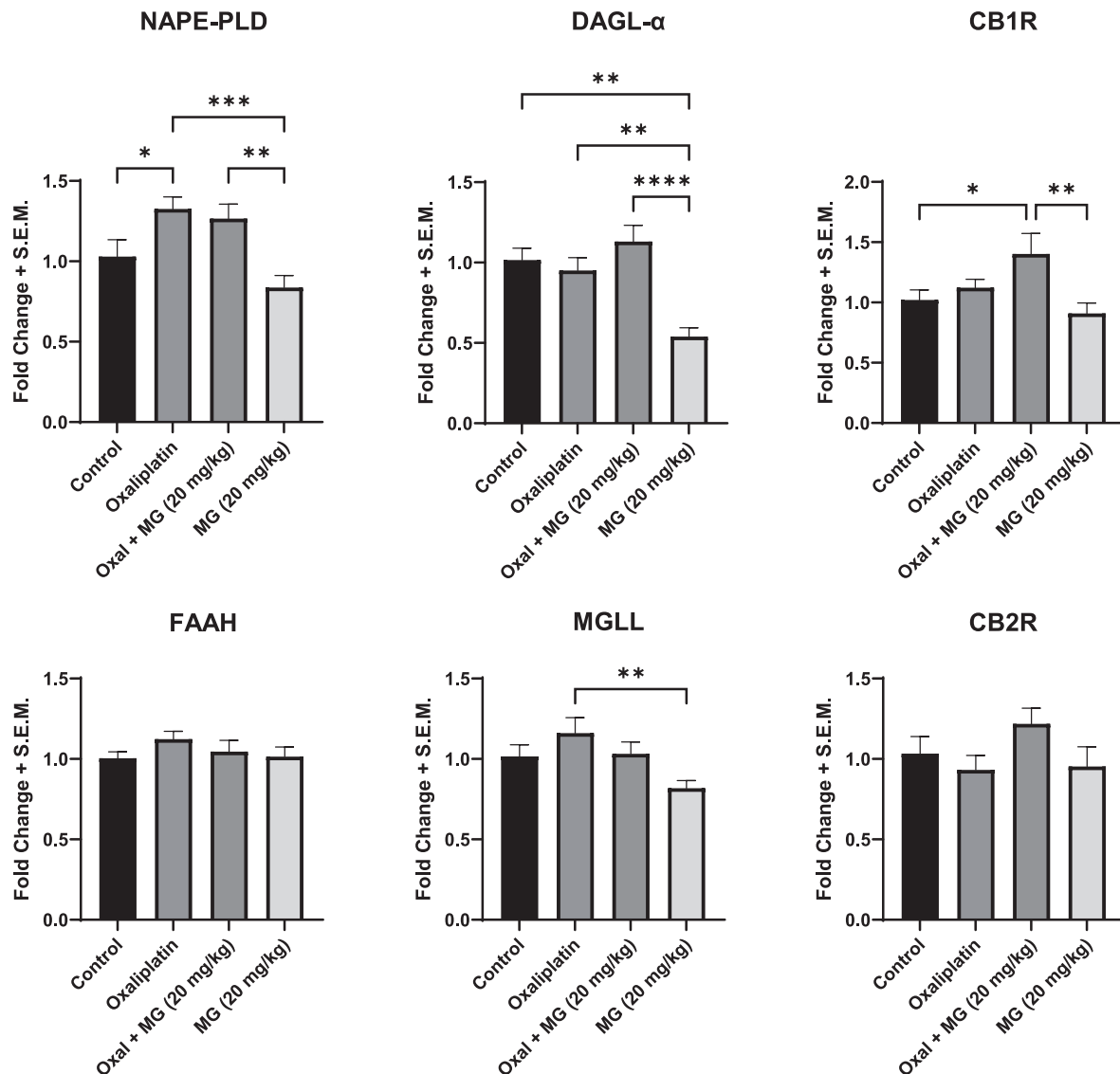


Fig. 6. Effects of oxaliplatin and MG exposure on lumbar spinal cord mRNA expression of cannabinoid receptors and metabolic enzymes of endocannabinoids. Lumbar spinal cords were harvested following a single injection of oxaliplatin and six consecutive days of MG exposure. mRNA expression of Nape-pld was significantly greater in oxaliplatin-treated mice compared to controls. Across all targets, no changes were detected between mice treated with oxal + MG and oxaliplatin alone. Mice repeatedly treated with MG alone exhibited significantly lower mRNA expression of Nape-pld, Dagl-α, Cnr1, and Mgl1 compared to either controls, oxaliplatin alone, or mice treated with oxal + MG. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. All groups male, $n = 7-12$. All data are represented as mean \pm SEM.

mechanisms contributing to this model-specific effect at the level of the spinal cord. To do this, we performed HPLC-MS/MS to investigate the influence of oxaliplatin and MG on the endocannabinoid lipidome in the lumbar spinal cord. Mice were treated with a single injection of oxaliplatin or vehicle, followed by six consecutive days of MG or vehicle. Lumbar spinal cords were harvested, lipids were extracted from tissue and levels of a variety of endocannabinoids and related lipid mediators were measured (Table 1; Supplementary Table 1). One-way ANOVA detected a significant main effect of treatment for 2-linoleoylglycerol (2-LG) [$F(3, 35) = 3.307$, $P < 0.05$], 2-oleoylglycerol (2-OG) [$F(3, 36) = 3.340$, $P < 0.05$], and docosahexaenoic acid [$F(3, 35) = 4.272$, $P < 0.05$]. Fisher's LSD revealed that the total abundance of 2-LG, linoleic acid, and eicosapentaenoic acid were significantly lower in oxaliplatin-treated mice compared to vehicle controls. A statistically non-significant trend was reported for docosahexaenoic acid in oxaliplatin-treated mice compared to controls ($P = 0.08$). No significant differences were detected for prostaglandins or *N*-acyl ethanolamines between controls and oxaliplatin or oxaliplatin + MG animals. Compared to oxaliplatin

alone, significant increases were detected in total abundance of *N*-oleoyl ethanolamine, anandamide, 2-OG, 2-LG, 2-arachidonylglycerol (2-AG), oleic acid, eicosapentaenoic acid, and docosahexaenoic acid in oxaliplatin + MG animals. MG treatment alone or in combination with oxaliplatin did not affect total abundance of prostaglandins compared to oxaliplatin alone. Trending, but statistically non-significant increases in arachidonic acid were found in mice treated with MG alone ($P = 0.08$) or in combination with oxaliplatin ($P = 0.06$) compared to oxaliplatin alone.

4. Discussion

4.1. MG and cannabinoid receptor expression and activation in CIPN

The present study marks the first to characterize the role of cannabinoid receptor signaling of a kratom alkaloid in a neuropathic pain model. We first determined whether the anti-allodynic effects of MG would be preserved in the absence of CB1 and CB2 receptors. In WT

Table 1
Effects of oxaliplatin and repeated MG exposure on lumbar spinal cord endocannabinoid lipidome. Lumbar spinal cords were harvested following a single injection of oxaliplatin and six consecutive days of MG exposure. Levels of endocannabinoids and related lipids and lipoamines were measured by HPLC-MS/MS. Analyzed data are represented in tabular format as heatmaps and illustrate the direction in fold change and significance. All groups male, $n = 9-10$.

Lipid Species	Treatments compared to Vehicle		
	Oxaliplatin	MG (20 mg/kg)	Oxaliplatin + MG (20 mg/kg)
N- acyl ethanolamine			
N- palmitoyl ethanolamine			
N- stearoyl ethanolamine			
N- oleoyl ethanolamine			
N- linoleoyl ethanolamine			
N- arachidonoyl ethanolamine			
N- docosahexaenoyl ethanolamine			
2-acyl-sn-glycerol			
2-palmitoyl-sn -glycerol			
2-oleoyl-sn -glycerol			
2-linoleoyl-sn -glycerol	↓		
2-arachidonoyl-sn -glycerol			
Free Fatty Acids			
Oleic Acid			
Linoleic Acid	↓↓	↓↓	
Arachidonic Acid			
Eicosapentaenoic Acid	↓		
Docosahexaenoic Acid	↓↓		
Prostaglandins			
PGE2			
PGF2α			

Lipid Species	Compared to Oxaliplatin	
	MG (20 mg/kg)	Oxaliplatin + MG (20 mg/kg)
N- acyl ethanolamine		
N- palmitoyl ethanolamine		↑
N- stearoyl ethanolamine		
N- oleoyl ethanolamine		↑↑
N- linoleoyl ethanolamine		
N- arachidonoyl ethanolamine		↑↑
N- docosahexaenoyl ethanolamine		
2-acyl-sn-glycerol		
2-palmitoyl-sn -glycerol		
2-oleoyl-sn -glycerol		↑↑
2-linoleoyl-sn -glycerol		↑↑
2-arachidonoyl-sn -glycerol		↑
Free Fatty Acids		
Oleic Acid		↑↑↑
Linoleic Acid		
Arachidonic Acid	↑	↑
Eicosapentaenoic Acid	↑	↑
Docosahexaenoic Acid		↑↑↑
Prostaglandins		
PGE2		
PGF2α		

mice, repeated MG exposure significantly prevented the development of oxaliplatin-induced mechanical allodynia, consistent with our previous findings [32,33]. MG produced an anti-allodynic effect in $Cnr1^{-/-}$ / $Cnr2^{-/-}$ mice on day 2 only. This effect may be due to compensatory mechanisms resulting from genetic deletion of cannabinoid receptors such as enhanced reliance on opioid-mediated analgesia, which contributes to MG's anti-allodynic effects [32,33]. This may increase susceptibility to tolerance, explaining the loss of an effect on days 5 and 7. However, mechanical thresholds did not significantly differ between MG-treated WT and $Cnr1^{-/-}$ / $Cnr2^{-/-}$ mice on days 5 and 7, suggesting only a partial loss of MG's therapeutic efficacy due to genetic deletion of cannabinoid receptors. Despite a lack of statistical significance, the magnitude in difference between percent baseline of paw withdrawal threshold between both MG groups across days 5 and 7 suggests biological significance (Supplementary Fig. 3). Oxaliplatin alone produced floor value mechanical thresholds in both WT and $Cnr1^{-/-}$ / $Cnr2^{-/-}$ mice, suggesting that the onset and duration of oxaliplatin-induced mechanical hypersensitivity is not influenced by cannabinoid receptor expression. While similar results have been reported in previous studies using CB1 or CB2 single knockout animals in paclitaxel CIPN models, this study marks the first use of cannabinoid receptor double knockout mice in a CIPN model, and the first to genetically manipulate cannabinoid receptor signaling in an oxaliplatin CIPN model. [46,53–55].

To characterize the individual contributions of CB1 and CB2 signaling to MG's anti-allodynic effects, mice were pretreated with SR141716 or SR144528 prior to MG exposure. The anti-allodynic effect of MG was completely blocked by 10 mg/kg of SR141716. Both 3 and 10 mg/kg of SR144528 significantly blocked the anti-allodynic effect of MG. These doses of SR144528 are also sufficient to block the anti-allodynic effect of cannabidiol (CBD), the CB2 agonist R, S-AM1241, and the MGLL inhibitors JZL184 and MJN110 in paclitaxel CIPN [43,44,46]. Contrarily, the lack of a significant effect of SR141716 at 3 mg/kg reported here contradicts previous findings [43]. However, this study used exclusively female mice, a paclitaxel CIPN model, and varied in its SR141716 dosing scheme. Overall, both CB1 and CB2 activation contribute to the ability of MG to prevent oxaliplatin-induced

mechanical hypersensitivity.

4.2. MG and cannabinoid receptor activation and expression in inflammatory pain

This was the first study to examine the effects of an individual kratom alkaloid in the formalin test, as previous studies utilized kratom methanolic extract [56]. MG routinely reduced nocifensive licking behavior in phase II, suggesting antinociceptive effects against persistent inflammatory pain. This antinociceptive effect of MG was not disrupted by SR144528 or SR141716, aligning with the only other study to date on cannabinoid mechanisms of MG-induced antinociception, in which the CB1 antagonist AM251 does not disrupt MG antinociception in the hot plate test [40]. MG significantly reduced nocifensive behavior in phase I in SR141716 and KO studies, but not SR144528 or capsazepine studies. We believe this is due to lower licking times produced by formalin alone in SR144528 and capsazepine studies, as mean licking times of MG alone in phase I remained steady throughout all formalin experiments. Surprisingly, the antinociceptive effect of MG was enhanced in $Cnr1^{-/-}$ / $Cnr2^{-/-}$ mice compared to WT in phase II. Less nocifensive behavior was observed in vehicle-treated $Cnr1^{-/-}$ / $Cnr2^{-/-}$ mice compared to vehicle WT in both phases, raising the possibility of compensatory mechanisms and potential epistatic effects as a consequence of genetic deletion of cannabinoid receptors [57]. Zimmer et al. reported that formalin induces less licking behavior in CB1 KO mice compared to WT, suggesting that mice lacking CB1 receptors are less sensitive to inflammatory pain [58]. In summary, these results suggest that MG is effective in treating persistent inflammatory pain in mice independent of cannabinoid signaling.

4.3. TRPV1 contributions to MG in neuropathic and inflammatory pain

The role of TRPV1 signaling in the pathophysiology of inflammatory and neuropathic pain has been well documented, and is activated by cannabinoid ligands such as CBD and AEA [35,36,51,59–62]. As such, capsaicin patches are used for several chronic neuropathic pain

conditions [28,63,64]. Capsazepine pretreatment blocked the anti-allodynic effect of MG in a CIPN model, but not MG's effect on formalin-induced pain. Aberrant TRPV1 signaling and expression has been documented at the level of the dorsal root ganglion (DRG) in CIPN [65,66]. Furthermore, presynaptic activity of TRPV1 is suppressed by noradrenaline and α 2-adrenergic receptors [67]. Given the contributions of adrenergic signaling to the efficacy of MG in acute and neuropathic pain states, MG may have an indirect effect on TRPV1 function and signaling. In fact, MG dose-dependently suppresses supraspinal TRPV1 protein expression in rats in an acetic acid-induced writhing test [68]. However, this has yet to be explored in the spinal cord or periphery, or in a CIPN model.

4.4. Impact of MG on cannabinoid metabolism and lipids

To improve our mechanistic understanding of the contribution of cannabinoid signaling to MG pharmacology, we tested the effects of MG on cannabinoid receptor-mediated signal transduction *in vitro*. cAMP production was chosen as our endpoint based on its prominent role in cannabinoid-dependent regulation of neuronal function, and the proposed contribution of adenylyl cyclase inhibition to the modulation of nociceptive signaling [69–71]. While MG did not impact CB1- or CB2-mediated inhibition of cAMP signaling, this does not entirely rule out the possibility of MG affecting other forms of cannabinoid receptor-mediated signal transduction [69]. Furthermore, these findings neglect the potential contributions of oxaliplatin, which increases cAMP response in rat DRG *in vitro* [72]. Therefore, we further explored possible cannabinoid-related mechanisms of MG in the context of CIPN. In the lumbar spinal cord, NAPE-PLD, an enzyme upstream of AEA biosynthesis, was the only target which was significantly affected by oxaliplatin. Similar findings have been reported in the lumbar DRG of oxaliplatin-treated male rats [73]. Interestingly, changes in FAAH and MGLL were not detected, despite the efficacy of select inhibitors in preclinical CIPN models [74–78]. Overall, oxaliplatin and MG appear to have minimal effects on the mRNA expression of endocannabinoid enzymes and cannabinoid receptors in the lumbar spinal cord.

Given the diminutive effect of oxaliplatin on the expression of cannabinoid receptors or endocannabinoid biosynthetic or hydrolytic enzymes, we shifted our focus towards endocannabinoid lipid mediators and related lipamines within the spinal cord. While oxaliplatin alone did not significantly disrupt AEA or 2-AG expression compared to controls, others have shown that 2-AG and AEA expression increases in the lumbar spinal cord of mice with CIPN [75,76]. However, these studies used C3H/HeN mice and a cisplatin CIPN model. Nonetheless, both 2-AG and AEA levels were significantly greater in oxaliplatin + MG mice compared to oxaliplatin alone, suggesting repeated MG exposure may increase endocannabinoid tone. Given that both AEA and 2-AG display endogenous agonist activity at CB1 and CB2 receptors and the established anti-allodynic efficacy of CB1 and CB2 agonists in preclinical CIPN models, MG may produce anti-allodynic effects through restoring endocannabinoid tone in the spinal cord [60,79]. Given that AEA and 2-AG levels were increased, but FAAH and MGLL levels were not affected in oxaliplatin + MG mice, MG may be increasing endocannabinoid tone through a mechanism independent of MGLL or FAAH inhibition. The widespread increase in a range of N-acyl ethanolamines, 2-acylglycerols and free fatty acids in oxaliplatin + MG, but not MG alone animals compared to oxaliplatin suggests that MG may be having an upstream effect on their biosynthesis in neuropathic pain states. One possibility is through phospholipase A2 (PLA₂), an enzyme which catalyzes fatty acid cleavage, and diminishes oxaliplatin-induced mechanical hypersensitivity in mice when exogenously administered as bee-derived PLA₂ (bvPLA₂) [80–82]. Further supporting this possibility is the adrenergic, serotonergic, and opioid-dependent mechanisms of bvPLA₂, which bears resemblance to MG. Expression of PLA₂ isoforms are disrupted in mouse DRG following acute oxaliplatin exposure, but long-term effects in the spinal cord remain unstudied [83].

4.5. Limitations

One limitation of the data presented is the strict assessment of stimulus-evoked nociceptive behaviors. The use of von Frey filaments to measure reflexive withdrawal responses can limit data interpretation, as these techniques may provoke hyperactivation of spinal reflexive circuits, and may not necessarily be due to a pain-like response [84]. Additionally, MG's mechanistic site of action remains elusive, as MG was administered intraperitoneally across all studies. Oral administration of kratom alkaloids has been studied in models of acute and chronic pain, but not yet CIPN [11,85,86]. Given the heavy emphasis on the effects of oxaliplatin and MG on cannabinoid molecular pharmacology at the level of the spinal cord, future studies utilizing intrathecal MG administration are warranted. Lastly, *in vivo* assessment of pharmacokinetics upon administering MG in combination with cannabinoid antagonists is warranted, as potential drug-drug interactions may help explain the discrepancies between *in vivo* and *in vitro* data.

5. Conclusions

Overall, our findings demonstrated that MG prevented the development of oxaliplatin-induced mechanical hypersensitivity through mechanisms involving cannabinoid receptor and TRPV1 activation. Oxaliplatin-induced depletion of select endocannabinoid lipids was prevented by repeated MG exposure, suggesting that MG's cannabinoid mechanisms in the context of CIPN may be due to an effect indirect of cannabinoid receptor activation. Additionally, our results suggest for the first time that MG is effective against persistent inflammatory pain.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lfs.2023.121878>.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Data availability

The data that supports the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical reasons.

Acknowledgements

This research was supported by NIDA awards P30DA013429 and T32DA007237.

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