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THC and gabapentin interactions in a mouse neuropathic pain model

Nicholas Atwal, Sherelle L. Casey, Vanessa A. Mitchell, Christopher W. Vaughan*

Pain Management Research Institute, Kolling Institute of Medical Research, Northern Clinical School, Royal North Shore Hospital, The University of Sydney, NSW, Australia

HIGHLIGHTS

- We examined interactions between tetrahydrocannabiniol (THC) and gabapentin in a mouse neuropathic pain model.
- THC and gabapentin synergistically reduced allodynia.
- Coadministration with gabapentin increased the therapeutic window of THC.
- Thus, THC may provide an adjuvant to current neuropathic pain medications.

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ABSTRACT

Clinical studies have shown that the major psychoactive ingredient of *Cannabis sativa* Δ9-tetrahydrocannabinol (THC) has some analgesic efficacy in neuropathic pain states. However, THC has a significant side effect profile. We examined whether the profile of THC could be improved by co-administering it with the first-line neuropathic pain medication gabapentin. This was done using the chronic constriction injury (CCI) model of neuropathic pain in C57BL6 mice. At 8 days post-CCI nerve injury, acute systemic administration of gabapentin produced a dose-dependent decrease in CCI-induced mechanical and cold allodynia, and increased motor incoordination. Coadministration of THC and gabapentin in a fixed-ratio dose-dependently reduced mechanical and cold allodynia, and produced all the side-effects observed for THC, including motor incoordination, catalepsy and sedation. Isobolographic analysis indicated that the ED_{50} for the THC:gabapentin induced reduction in allodynia was 1.7 times less than that predicted for an additive interaction. The therapeutic window of combination THC:gabapentin was greater than that for THC alone. These findings indicate that gabapentin synergistically enhances the anti-allodynic actions of THC and improves its therapeutic window. Thus, THC may represent a potential adjuvant for neuropathic pain medications such as gabapentin.

1. Introduction

Neuropathic pain is a particularly severe form of chronic pain which arises from a lesion or disease affecting the somatosensory system (Jensen et al., 2011). Current first-line treatments for neuropathic pain include anticonvulsants such as gabapentin and pregablin, antidepressants, and topical lidocaine (Dworkin et al., 2010). Current therapies, however, have variable effectiveness and produce side effects which reduce compliance and satisfaction (Baron et al., 2010; Dworkin et al., 2010). The major psychoactive ingredient of *Cannabis sativa*, Δ9-tetrahydrocannabinol (THC), has been proposed as an alternative treatment for neuropathic pain sufferers. While there is evidence that THC has efficacy in neuropathic pain states (Abrams et al., 2007; Ellis et al., 2009; Ware et al., 2010; Wilsey et al., 2013), systematic reviews indicate that this efficacy is variable and that side-effects are problematic (Boychuk et al., 2015; Nugent et al., 2017; Whiting et al., 2015).

A number of studies have shown that THC has analgesic efficacy in animal models of neuropathic pain (Comelli et al., 2008; De Vry et al., 2004a, 2004b; Deng et al., 2015; Harris et al., 2016; Williams et al., 2008). Cannabinoids, however, produce a spectrum of side-effects in rodents including catalepsy, sedation, motor and cognitive impairment (Rahn and Hohmann, 2009). In animals which have undergone a neuropathic pain model, the THC induced side-effects are observed at doses similar to those at which it reduces allodynia (Casey et al., 2017). This suggests that while THC has potential as a neuropathic pain medication, its therapeutic window needs to be improved.

* Corresponding author. Pain Management Research Institute, Kolling Institute of Medical Research, Northern Clinical School, University of Sydney at Royal North Shore Hospital, St Leonards, NSW, 2065, Australia..
E-mail address: chris.vaughan@sydney.edu.au (C.W. Vaughan).
URL: http://www.pmri.med.usyd.edu.au (C.W. Vaughan).

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Current first-line treatments for neuropathic pain are often used in combination to enhance analgesic efficacy and the therapeutic window (Dworkin et al., 2010). If the therapeutic window of cannabinoids can be improved through combinational therapy they may represent useful adjuvants for neuropathic pain. For example, a small scale clinical trial found that THC increased the analgesic effect of opioids in chronic pain sufferers (Abrams et al., 2011), although the extent and nature of this drug interaction is unknown. Drug interactions can be more precisely examined in animal studies using an isobolographic approach which can quantify additive, sub-additive and synergistic drug behaviour (Tallarida, 2006). For example, isobolographic studies have shown that THC and synthetic cannabinoid receptor agonists act synergistically with the non-psychoactive cannabis constituent cannabidiol, morphine, noradrenaline reuptake inhibitors and COX inhibitors to reduce allodynia in animal neuropathic pain models (Casey et al., 2017; Grenald et al., 2017; Gunduz et al., 2016; Kazantzis et al., 2016; King et al., 2017). However, the effect THC in combination with current first-line neuropathic pain medications, such as gabapentin, has not been examined. In this study we examined whether the anti-allodynic and side effect profiles of THC were altered by coadministration with gabapentin in an animal model of neuropathic pain.

2. Material and methods

2.1. Animals

Experiments were performed on adult C57BL/6 male mice during the day cycle using the ARRIVE and 'NH&MRC Code of Practice for the Care and Use of Animals in Research in Australia' guidelines. All experimental procedures described below were carried out as approved by the Royal North Shore Animal Care and Ethics Committee (protocol number RESP-16-262). Mice were housed individually in ventilated cages under controlled light (12 h light-dark cycles) and temperature (23 ± 1 °C, 70% humidity) with *ad libitum* access to water and food pellets.

2.2. Pain model

All surgical procedures were conducted under isoflurane anaesthesia (2%) saturated in O_2. Nerve injury induced neuropathic pain was produced in mice using the chronic constriction injury (CCI) model (Bennett and Xie, 1988). Blunt dissection of the biceps femoris muscle was used to expose the left hind sciatic nerve. A segment of the sciatic nerve proximal to the sciatic trifurcation was freed from surrounding tissues and two ligatures (7–0 chromic gut) were loosely tied around the sciatic nerve 2 mm apart. The muscle layer was closed using 6.0 silk sutures and the skin layer was sealed using glue. Animals were euthanased if post-operative complications arose.

2.3. Drugs and administration

Δ9-Tetrahydrocannabinol was obtained from National Measurement Institute (Lindfield, Australia) and THCPharm (Frankfurt, Germany); gabapentin was from Cayman Chemicals (Ann Arbor, USA); all other reagents from Sigma-Aldrich (Castle Hill, Australia). On the testing day, mice received a subcutaneous injection of either a drug or vehicle under brief 2% isoflurane anaesthesia. Gabapentin was prepared in saline while THC was prepared in 2% randomly methylated beta-cyclodextrin (RAMEB), 15% dimethylsulfoxide (DMSO) and 5% Tween-80 in saline to ensure dissolution. The THC:gabapentin combination vehicle was the same as that for THC. The vehicles used in drug treatment groups served as a negative control. All agents were injected at a volume of 0.1 mL per 10 g body weight. Researchers were blinded to all drug treatment groups (n = 6, per treatment group).

2.4. Behavioural testing

All behavioural testing, including allodynia and side-effect measurements, were performed under low level red light (< 3 lux), as described previously (Adamson Barnes et al., 2016; Anderson et al., 2014; Casey et al., 2017; Kazantzis et al., 2016). Mechanical allodynia was assessed by measuring the mechanical paw withdrawal threshold (PWT) using a series of von Frey filaments (0.2–8.5 g). Each von Frey hair was gently pushed onto the plantar surface of the CCI paw. The presence or absence of pain-like behaviours (paw lifting, licking, shaking) was recorded. The mechanical PWT was calculated using a simplified up-down tracking measure (Bonin et al., 2014). Cold allodynia was measured using the acetone test. 20 μL of acetone was administered directly onto the plantar surface of CCI paw using a pipette to induce evaporative cooling of the hind paw. The frequency of pain-like behaviours was counted over a 1-min time period.

Side-effects including motor-incoordination, catalepsy and sedation were measured using the rotarod, bar test and dark open field. Mice were placed on the rotarod which accelerated (5–30RPM) over a 300 s time period and the time taken for mice to fall off was recorded. For the bar test, the front paws of the mouse were placed on a horizontal bar suspended 4.5 cm above the ground inside an enclosed box, and the time taken for the mouse to remove both front paws from the bar was recorded. For the dark open field, mice were placed in an enclosed open top arena (40 × 40 × 40 cm) and an overhead camera recorded the behaviour of the mouse for 2.5 min. Activity was measured as the total number of grid crossings (4 × 4 grid).

2.5. Protocol

After the mice were received, initial acclimatisation to all behavioural testing devices was conducted; except for the open field as this test requires a degree of novelty. After acclimatisation, pre-CCI surgery baseline values of all measures (except open field) were obtained. 8 days post-CCI surgery, pre-drug behavioural measurements were conducted. The drug, or vehicle was subsequently injected, then post-drug treatment behavioural testing was performed at fixed time points (see below). Mice only underwent the open field test once, after drug injection, in order to retain environmental novelty.

For the time course experiments, pain and side-effect testing was performed prior to, then at 0.5, 1, 1.5, 2, 3, 4, 5 and 6 h after drug administration. Only acetone responses and rotarod latency were assessed in this experiment because the relatively short post-drug testing intervals precluded assessment of all pain and side-effect tests. For the dose-response experiments all pain and side-effect assays were assessed. In these experiments, pain and side-effect testing was performed prior to, then at the time of peak effect (1.5 h after drug administration, see results). For the THC dose response experiments, only curve fits are shown because, while some new data was obtained for 2 intermediate doses, most of this data was from our recent study (Casey et al., 2017).

2.6. Analysis

For the time course experiments raw data was analysed using repeated two-way measures ANOVA (Prism, Graphpad Software, La Jolla, USA) with the factors drug treatment group (between-subjects factor) and time post-drug injection (within-subjects factor). Post-hoc comparisons between drug and vehicle were made at individual time points using the Bonferroni adjustment for multiple comparisons.

For the dose response experiments, behavioural testing data was normalised, except for the open field test, as this test was only conducted after drug administration. A normalised score of the percentage of the maximum possible effect (MPE) was obtained as follows: for mechanical PWT and bar latency MPE (%) = 100 *(Post-Drug – Pre-Drug)/(Cut-off – Pre-Drug), with cut-off values of 6.84 g and 120 s, respectively; for acetone responses and rotarod latency MPE (%) = 100

* (Pre-Drug – Post-Drug)/(Pre-Drug). A sigmoidal function was fit to the dose response data for each drug and assay to obtain the maximal effect (MAX), ED50 and Hill slope (p) (Prism). This was calculated using the following equation:

$$Effect = E_{Max}\frac{Dose^p}{[Dose^p + ED_{50}^p]} \quad (1)$$

Isobolographic analysis was used to assess interactions between THC and gabapentin. The predicted additive effect of THC and gabapentin, E(a,b), was calculated from individual dose response profiles using an approach makes no assumptions about the maximal effect and Hill slopes of the two drug (Kazantzis et al., 2016; Tallarida, 2006).

$$E(a,b) = \frac{E_B\left(b + \frac{C_B}{k^{1/p}}\right)^p}{\left[\left(b + \frac{C_B}{k^{1/p}}\right)^p + C_B^p\right]} \quad (2)$$

, *where* $k = \frac{E_B}{E_A}\left(1 + \frac{C_A^q}{a^q}\right) - 1$at doses a and b for drugs A and B with maximal effects of E_A and E_b (where $E_b > E_A$), ED_{50}s of C_A and C_B, and Hill slopes of p and q. The experimental combinational therapy dose response curve was then compared to the theoretical predicted additive effect of THC and gabapentin obtained from equation (2) using a modified *t*-test to compare data at specific doses on the experimental and theoretical dose response curves.

From this the non-linear isobole which describes the relationship between the drug doses a and b, at a specified effect level B_i, was calculated as follows:

$$b = B_i - \frac{C_B}{\left[\frac{E_B}{E_A}\left(1 + \frac{C_A^q}{a^q}\right) - 1\right]^{1/p}} \quad (3)$$

The degree of synergy, or interaction index, was calculated as the ratio of the experimental ED_{50} for the drug combination compared to the ED_{50} for its predicted additive effect. The therapeutic index (T.I.) was calculated by the mean ED_{50} of the side-effects divided by the mean ED_{50} of anti-allodynia. Data is shown as the mean and s.e. mean, or the 95% confidence interval (C.I., for ED_{50}s).

3. Results

3.1. Time course of action of gabapentin and THC

At 8 days post-CCI surgery there was an increase in acetone responses and a reduction in rotarod latency compared to the baseline pre-CCI values (Fig. 1, t(18) = 15.1, 2.8, p < 0.0001, 0.05). We first examined the time course of gabapentin and THC to establish the time of peak effect for subsequent dose response experiments. In these experiments we used near-maximal analgesic doses of gabapentin (100 mg kg^{-1}) and THC (17.8 mg kg^{-1}) which were 4.3 and 4.7 times their respective ED_{50} values (Fig. 2, Table 1) (Casey et al., 2017). At 8 days post-CCI, there was a significant interaction between treatment groups and time for acetone responses (F (24, 160) = 7.8, p < 0.0001) and rotarod latency (F (24, 160) = 4.4, p < 0.0001).

Gabapentin produced a significant reduction in acetone responses at 1–4 h post-injection compared to the pre-injection level (Fig. 1a, P < 0.0001–0.05). THC produced a significant reduction in acetone responses at 0.5–4 h post-injection compared to the pre-injection level (Fig. 1a, P < 0.0001–0.05). Gabapentin also produced a significant reduction in rotarod latency at 1.5–2 h post-injection compared to the pre-injection level (Fig. 1b, P < 0.01–0.05). THC also produced a significant reduction in rotarod latency at 0.5–5 h post-injection compared to the pre-injection level (Fig. 1b, P < 0.0001–0.05). By contrast, vehicle did not have a significant effect on acetone responses, or rotarod latency at any post-injection time point (Fig. 1a, b, p > 0.05).

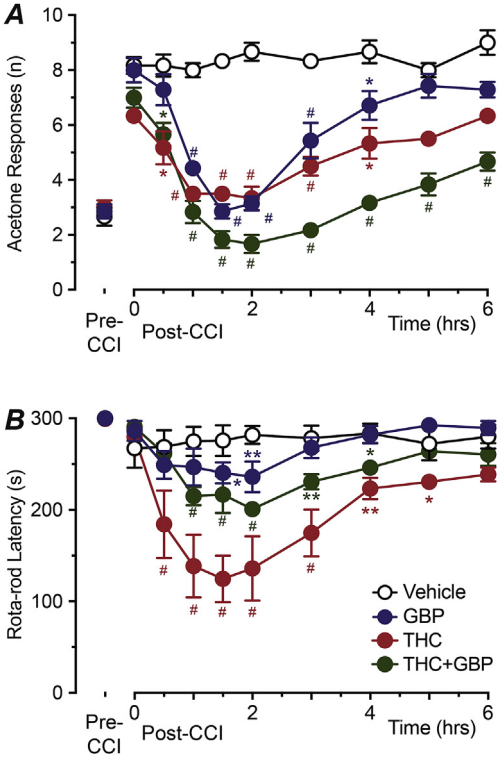

Fig. 1. Time course of action of Gabapentin and THC, alone and in combination. Time plots of effect of gabapentin (100 mg kg^{-1}), THC (17.8 mg kg^{-1}), THC plus gabapentin (30 mg kg^{-1}) and matched vehicle on (A) acetone responses and (B) rotarod latency (n = 6 per treatment group). Mice received a subcutaneous injection at time 0 h, 8 days post-CCI surgery; pre-CCI data is also display. *, ** and # denote p < 0.05, 0.01 and 0.0001 versus the pre-injection time point 0, for each treatment group.

3.2. Dose-response profiles of gabapentin

We next obtained dose response curves for the effect of acute administration of gabapentin (dose range of 1–562 mg kg^{-1}, at 0.25–0.5 decade intervals) on all allodynia and side-effect assays, testing at the average time of peak effect determined in the above time course experiment (1.5 h). Gabapentin produced a dose dependent attenuation of CCI induced mechanical and cold allodynia, with ED_{50}s of 18 and 28 mg kg^{-1} (Fig. 2a and b, Table 1). The ED_{50} of gabapentin for mechanical PWT was significantly lower than that for acetone responses (F (1,10) = 7.4, p < 0.05). The maximal effect of gabapentin on mechanical PWT was also significantly greater than that for acetone responses (Table 1, F (1,10) = 12.04, p < 0.01).

As the aim of the study was to examine gabapentin interactions with THC, we also obtained dose response data for cannabinoid-like side-effects. Gabapentin produced a dose dependent decrease in rotarod latency, although the ED_{50} and E_{max} could not be accurately estimated because a maximal effect was not obtained over the range of doses tested (Fig. 2c, Table 1). Gabapentin had no effect on bar latency, or open field crossings over the range of doses tested (Fig. 2d and e, 1–562 mg kg^{-1}). The therapeutic window of gabapentin was estimated to be greater than 20 for the measures of anti-allodynia and side-effects used in this study (Table 1). THC increased mechanical PWT and decreased acetone responses with ED_{50}s of 4.3 and 3.2 mg kg^{-1} (Fig. 3, Table 1) (Casey et al., 2017).

3.3. Anti-allodynic effects of combination THC:gabapentin

We next examined the acute anti-allodynic effects of gabapentin when coadministered with THC (total THC plus gabapentin dose range

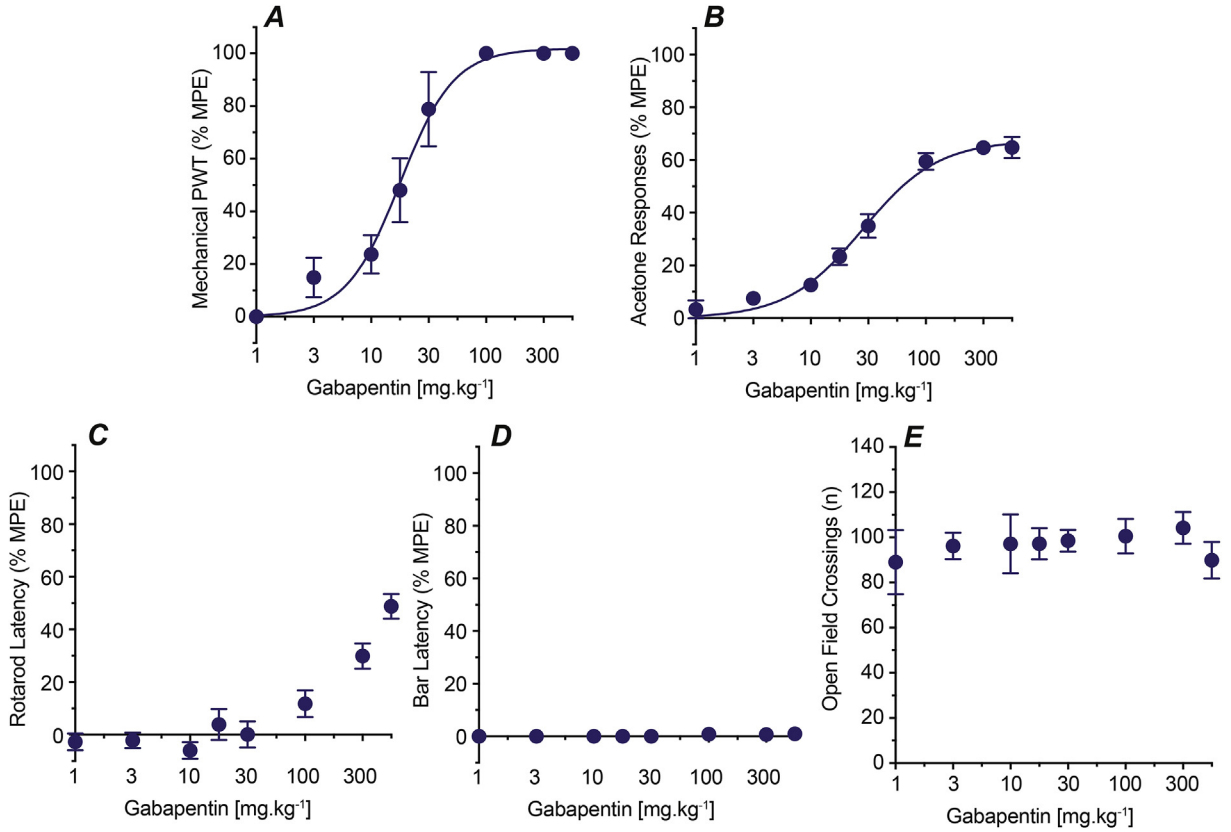

Fig. 2. Gabapentin produces a dose dependent reduction in mechanical and cold allodynia. The effect of gabapentin on (A) mechanical paw withdrawal threshold (Mech PWT), (B) acetone responses, (C) rotarod latency, (D) bar latency and (E) open field crossings responses (n = 6 per treatment group). A sigmoidal curve was fit to the data in (A)–(B). Data in (A)–(D) are shown as maximal possible effect (% MPE); whereas raw values are shown in (E).

of 0.3–300 mg kg^{-1}, at 0.5 decade intervals). For the combination experiments, gabapentin and THC were coadministered in a 1:1 fixed ratio by their anti-allodynic ED_{50}s (averaged across mechanical and cold allodynia) which equated to a ratio of 6.6:1 by weight. Using an isobolographic model which accounts for drugs having differing maximal effects and Hill slopes, combination THC:gabapentin was predicted to have ED_{50}s of 13.0 and 13.7 mg kg^{-1} for mechanical and cold allodynia, respectively, if they interacted in a simple additive manner (Fig. 3a and b, Table 1).

The fixed ratio combination of THC:gabapentin produced a dose dependent increase in mechanical PWT and decrease in acetone responses, both of which were left shifted compared to their theoretical predicted additive dose response curves (Fig. 3a and b, Table 1). In addition, the maximal effect of combination THC:gabapentin on acetone responses was greater than that predicted for an additive interaction (Fig. 3b, Table 1). Thus, combination THC:gabapentin had a significantly greater effect on mechanical PWT than that predicted for an additive interaction at doses ranging from 1 to 30 mg kg^{-1} (Fig. 3a,

Table 1
Dose response curve characteristics of THC, gabapentin and their combination.

	Anti-allodynia		Side-Effects			T.I.
	Mechanical PWT	Acetone Responses	Rotarod Latency	Bar Latency	Open Field Crossings	
THC						
ED_{50}	4.3 (3.3–6.1)	3.2 (1.9–9.1)	13.2 (10.1–17.3)	24.2 (23.4–25.1)	6.5 (4.0–9.9)	4.0 (1.5–7.6)
E_{MAX}	93 (5)	74 (7)	72 (17)	101(4)		
Hill Slope	1.8 (0.3)	1.8 (0.3)	1.9 (0.4)	3.8 (0.2)	1.5 (0.3)	
Gabapentin						
ED_{50}	17.9 (14.1–22.7)	28.2 (22.0–36.1)	> 562	> 562	> 562	> 20
E_{MAX}	102 (4)	67 (3)	N.D.	N.D.	N.D.	
Hill Slope	1.9 (0.3)	1.3 (0.2)	N.D.	N.D.	N.D.	
THC + Gabapentin: predicted additive						
ED_{50}	13.0	13.7	N.D.	N.D.	N.D.	N.D.
E_{MAX}	100	74				
Hill Slope	1.8	1.5				
THC + Gabapentin: Experimental						
ED_{50}	6.7 (5.5–8.2)	9.0 (5.7–14.3)	46.4 (32.1–70.7)	86.1 (85.4–86.9)	36.4 (31.7–44.2)	7.3 (4.0–12.9)
E_{MAX}	101 (2)	100 (3)	73 (6)	39 (1)		
Hill Slope	2.3 (0.3)	1.1 (0.2)	2.4 (0.7)	3.7 (0.1)	3.0 (0.7)	

*ED_{50} (mg·kg^{-1}), E_{MAX} (as % MPE, or number of crossings for the open field test), N.D (not determined) and values are shown as the mean (± s.e. mean, or 95% C.I.).

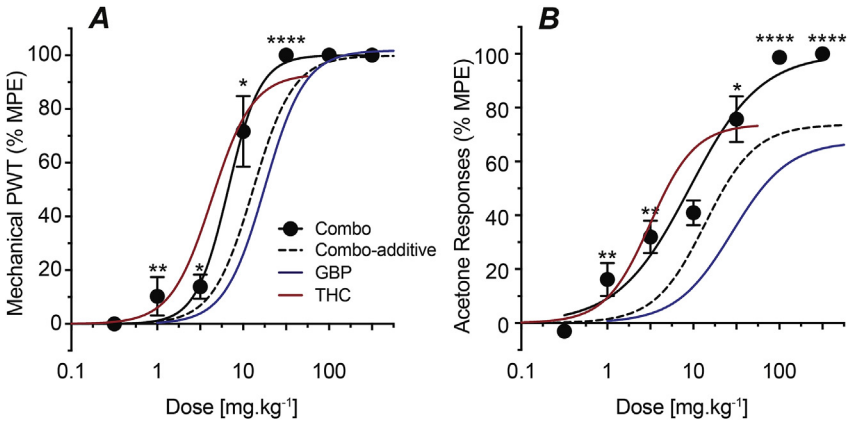

Fig. 3. Combination THC:gabapentin dose dependently reduces mechanical and cold allodynia. Dose response curves for the effect of THC coadministered with gabapentin in a 1:1 ED_{50} fixed ratio on (A) mechanical paw withdrawal threshold (PWT) and (B) acetone responses. Data is shown for experimentally obtained combination THC:gabapentin (black circles). Sigmoidal curve fits are shown for experimentally obtained combination THC:gabapentin (black solid line), THC (red line, which includes some data from Casey et al. (2017)) and gabapentin (blue line, from Fig. 2), and for the predicted additive effect of combination THC:gabapentin (black dotted line). Each data point represents n = 6 and error bars denote mean ± s.e mean. *, ** and **** represent p < 0.05, 0.01 and 0.0001 for experimental combination THC:gabapentin versus predicted theoretical additive effects.

p < 0.05–0.0001). Combination THC:gabapentin also had a significantly greater effect on acetones responses than that predicted for an additive interaction at lower (1–3 mg kg^{-1}) and higher dose ranges (100–300 mg kg^{-1}) (Fig. 3b, p < 0.05–0.0001).

Isobolograms were obtained to determine the degree of anti-allodynic THC:gabapentin synergy for mechanical PWT and acetone responses. The isoboles for both mechanical PWT and acetone responses were non-linear and the 50% effect level isoboles did not intersect the axes at the individual drug ED_{50}s (Fig. 4a and b). The experimentally obtained THC:gabapentin combination ED_{50} for mechanical PWT was similar to that predicted to produce a 22% reduction in allodynia if the interaction was purely additive (Fig. 4a). Likewise, the experimentally obtained THC:gabapentin combination ED_{50} for acetone responses was similar to that predicted to produce a 25% reduction in allodynia if the interaction was purely additive (Fig. 4b).

3.4. Side-effect profile of combination THC:gabapentin

In the above animals we also examined whether the THC:gabapentin combination produced cannabinoid-like side-effects. THC had ED_{50}s of 13, 24 and 6.5 mg kg^{-1} for rotarod latency, bar latency, and open field crossing, with a therapeutic window of 4.0 (Fig. 5, Table 1) (Casey et al., 2017). Combination treatment with THC:gabapentin in the above fixed ratio produced a dose dependent decrease in rotarod latency and open field crossings, with ED_{50}s of 46 and 36 mg kg^{-1}, and an increase in bar latency, with an ED_{50} of 86 mg kg^{-1} (Fig. 5a–c, Table 1). The therapeutic index of combination THC:gabapentin was 7.3 when averaged over all allodynia and side-effect assays (Table 1).

An isobolographic analysis for the side-effects could not be performed because of the low levels of cannabinoid-like side-effects induced by gabapentin (Fig. 2c – e). Instead, the influence of gabapentin on the THC induced side-effects was determined by comparing the side-effect profiles of the THC component of combination THC:gabapentin to that of THC when administered alone (Fig. 5a – c). For rotarod latency, the ED_{50} of the THC component of THC:gabapentin combination (5.9 mg kg^{-1}, 95% C.I. = 3.9–9.2 mg kg^{-1}) was significantly less than that for THC when administered alone (Fig. 5a, Table 1, F(1, 12) = 14.9, p < 0.01). For bar latency, the ED_{50} of the THC component of THC:gabapentin combination (11.4 mg kg^{-1}, 95% C.I. = 11.3–11.5 mg kg^{-1}) was less than that for THC alone (Fig. 5b, Table 1, F(1, 12) = 141, p < 0.0001). However, the maximal effect of the THC component of THC:gabapentin combination on bar latency (39.0 ± 0.1% MPE) was less than that for THC alone (Fig. 5b, Table 1, F(1, 12) = 98, p < 0.0001). Finally, the ED_{50} of the THC component of THC:gabapentin combination for open field crossings (4.8 mg kg^{-1}, 95% C.I. = 4.2–5.8 mg kg^{-1}) was not significantly different to that for THC alone (Fig. 5a, F(1, 12) = 1.6, p > 0.05).

3.5. Time course of action of combination THC:gabapentin

To put the combination THC:gabapentin data into perspective we examined its time course of action at a dose that was predicted to produce sub-maximal analgesia if the interaction was additive (30 mg kg^{-1}, 2.2x the predicted ED_{50} dose). The THC:gabapentin combination produced a significant reduction in acetone responses at 1–6 h post-injection compared to the pre-injection level (Fig. 1a, P < 0.0001–0.05). The THC:gabapentin combination also produced a significant reduction in rotarod latency at 1–4 h post-injection compared to the pre-injection level (Fig. 1a, P < 0.0001–0.05). The reduction in acetone responses produced by THC:gabapentin was greater than that for THC alone at 1.5–6 h post-injection (Fig. 1a, p < 0.001–0.05). By contrast, the reduction in rotarod latency produced by the THC:gabapentin combination was less than that for THC alone at 0.5–2 h post-injection (Fig 1b and 0.01–0.05).

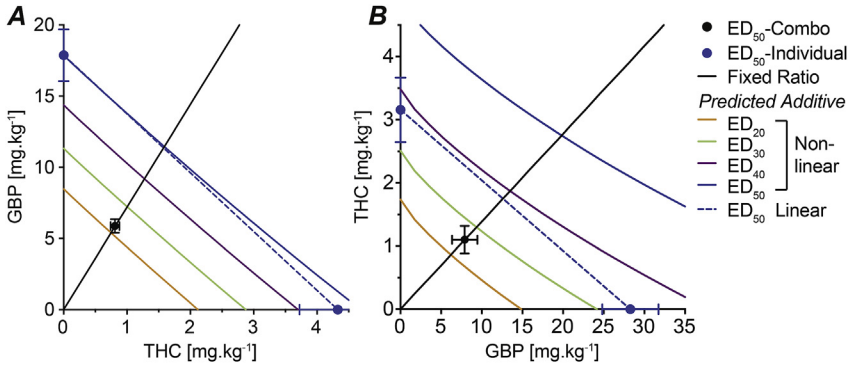

Fig. 4. Isoboles for combination THC:gabapentin effects on allodynia. Isoboles for the effect of THC coadministered with gabapentin on (A) mechanical paw withdrawal threshold and (B) acetone responses. The experimental combination ED_{50} (black circle) is shown along a continuum of potential fixed ratio effects (black line). Theoretical isoboles for predicted 20–50% effect levels are shown for comparison (solid colour lines) using a model which makes no assumptions about maximal drug effects or Hill slopes. Theoretical isobole for 50% effect level are also shown (blue dotted line) using a model which assume both drugs have similar maximal effects and Hill slopes of unity; with the individual ED_{50}s for THC and gabapentin (blue circles).

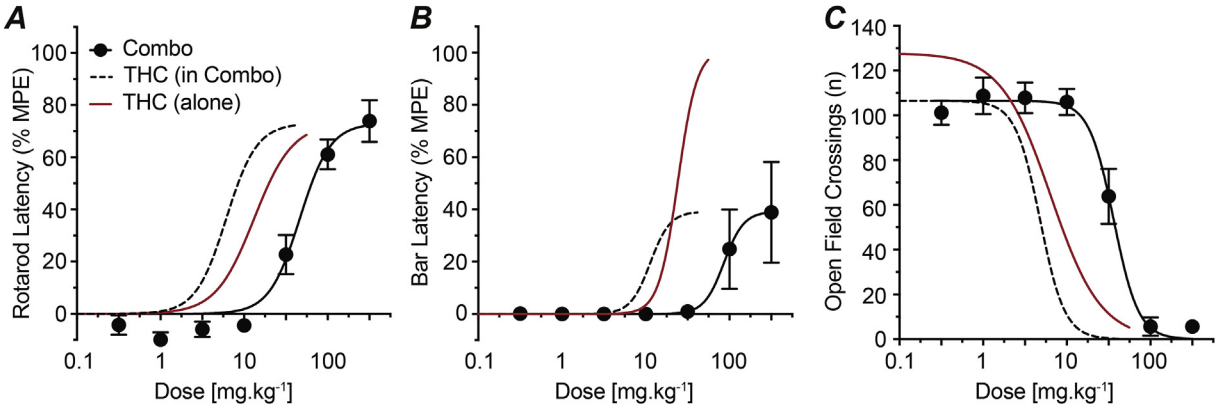

Fig. 5. Side-effect profiles of combination THC:gabapentin. The effect of combination THC:gabapentin (Combo) on (A) rotarod latency, (B) bar latency and (C) open field crossings. Also shown is the effect of THC alone (THC-Alone, red, curve fit of data some of which is from Casey et al. (2017)), and the THC component of the THC:gabapentin combination (THC-In Combo, calculated as the dose of THC in the 1:1 ED_{50} ratio combination).

4. Discussion

This study has demonstrated that THC and gabapentin act synergistically to reduce the allodynia induced by nerve injury in mice. The side-effects produced by THC were less affected by coadministration with gabapentin. These findings demonstrate that gabapentin enhances the therapeutic window of THC, and improves its anti-allodynic potency and efficacy in a mouse neuropathic pain model.

In the present study, acute systemic administration of gabapentin produced a dose dependent reduction of the mechanical and cold allodynia induced by the sciatic nerve chronic constriction injury model of neuropathic pain. The time course of the acute gabapentin induced reduction in mechanical and cold allodynia and its dose-response profile were similar to that reported in a range of neuropathic pain models (Câmara et al., 2015; Coderre et al., 2005; Gustafsson and Sandin, 2009; Kusunose et al., 2010; Lindner et al., 2006). Interestingly, gabapentin had a greater efficacy in reducing mechanical, compared to cold allodynia, as we have previously observed for THC (Casey et al., 2017). While gabapentin did not produce catalepsy or sedation, as measured by the bar and dark open field tests, it produced dose dependent motor-incoordination at higher doses, similar to that reported others (Gustafsson and Sandin, 2009; Lindner et al., 2006; Mixcoatl-Zecuatl et al., 2008). Together this confirms that gabapentin has a relatively high anti-allodynic efficacy and therapeutic window. It might be noted that gabapentin also produces cognitive deficits, such as in the Morris water maze test, and these were not examined in the present study (Lindner et al., 2006).

When gabapentin and THC were coadministered in a 1:1 ratio by ED_{50} (6.6:1 by weight), they produced a dose-dependent reduction in mechanical and cold allodynia. Using isobolographic analysis, it was found that the THC:gabapentin induced reduction in allodynia was synergistic. Indeed, the ED_{50} for combination THC:gabapentin induced reduction in allodynia was 1.7 (1.4–2.1) times less than that predicted if the two drugs acted in a simple additive manner, although the degree of synergy was greater for mechanical compared to cold allodynia. Furthermore, while THC and gabapentin only partly reduced cold allodynia when administered alone, they produced a complete reversal when administered in combination. This demonstrates that combination treatment increases both the potency and efficacy of gabapentin and THC. While the interaction between gabapentin and THC has not been examined previously in a neuropathic pain model, it might be noted that each of these act synergistically with a number of other agents to reduce allodynia (Casey et al., 2017; Espinosa-Juarez et al., 2016; Hama and Sagen, 2010; Hayashida and Eisenach, 2008; King et al., 2017; Miranda et al., 2015, 2016, 2017; Mixcoatl-Zecuatl et al., 2008).

The potential benefits of analgesic synergy between THC and gabapentin would be circumvented if there was also side-effect synergy. In the present study, the THC:gabapentin combination produced dose dependent catalepsy, sedation and motor incoordination. Thus, administering gabapentin in combination with THC introduced the side-effects associated with latter. Side-effect synergy could not be assessed using the isobolographic approach in the present study because a complete dose response profile for gabapentin side-effects was not obtained. Instead, potential interactions were assessed by comparing the side-effects produced by THC when administered alone to that in combination with gabapentin. It was found that coadministration with gabapentin produced subtle changes in the THC induced side-effects. Thus, gabapentin coadministration increased the potency of THC induced motor incoordination and catalepsy, but not sedation. Conversely, coadministration with gabapentin reduced the magnitude of THC induced catalepsy. Thus, only some of the side-effects of THC were enhanced when it was administered in combination with gabapentin. Even taking this into account, it was found that the therapeutic window of the THC:gabapentin combination (7.3) was greater than that for THC when administered alone (4.0) (Casey et al., 2017). Furthermore, when gabapentin and THC were coadministered at a predicted submaximal analgesic dose it produced a greater and longer lasting reduction in cold allodynia that THC alone. Interestingly, this dose of combination THC:gabapentin produced a lesser disruption of motor performance than THC alone. Together, these findings indicate that the potential benefits of THC in a neuropathic model are not reduced by negative side-effect interactions when used in combination with the current first-line neuropathic pain medication gabapentin. Finally, it should be noted that gabapentin is administered chronically and that future animal studies would need to examine whether the anti-allodynic efficacy and therapeutic window of the THC:gabapentin combination are maintained during long-term treatment.

In summary, this study has demonstrated that THC and gabapentin synergistically to attenuate allodynia in a nerve injury induced neuropathic pain model. This led to an enhancement of the therapeutic window the therapeutic window of THC. Thus, THC may represent a potential adjuvant to current first-line medications in the treatment of neuropathic pain.

Conflicts of interest

All authors declare that they have no conflicts of interest

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