Analysis of the anti-allodynic effects of combination of a synthetic cannabinoid and a selective noradrenaline re-uptake inhibitor in nerve injury-induced neuropathic mice

O. Gunduz, R.D. Topuz, C.H. Karadag, A. Ulugol

Department of Medical Pharmacology, Faculty of Medicine, Trakya University, 22030 Edirne, Turkey

Correspondence
Ozgur Gunduz
E-mail: gunduz_ozgur@yahoo.com;
ozgur@gunduz@trakya.edu.tr

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Abstract

Background: Combining drugs not only reduces specific adverse effects of each of the drug at a higher dose but also may lead to enhanced efficacy. Tapentadol is a recently discovered analgesic possessing μ-opioid receptor agonism and noradrenaline re-uptake inhibition in a single molecule. Taking into consideration, the pharmacological similarities between opioids and cannabinoids, we assumed that combination of cannabinoids with noradrenaline re-uptake inhibitors might also be effective. We therefore aimed to determine whether combining 1:1, 1:3 and 3:1 fixed ratios of the synthetic cannabinoid WIN 55,212-2 and the selective noradrenaline re-uptake inhibitor maprotiline exert anti-allodynic synergy on nerve-injured neuropathic mice.

Methods: Partial right ligation of the sciatic nerve was made in mice; on pre-operative and post-operative 15 days basal mechanical allodynia, cold allodynia and motor function were assessed using von Frey filaments, hot/cold plate and rotarod apparatus.

Results: Mechanical and cold allodynia developed in all groups on post-operative 15 days. Development of cold allodynia was statistically significant in all groups (p < 0.05); therefore, cold allodynia was used in combination studies. As shown by isobolographic analysis, interactions of 1:1 and 3:1 ratios of WIN 55,212-2; maprotiline combinations were supra-additive, whereas 1:3 ratio was sub-additive.

Conclusions: Overall, our data suggest that combination of a cannabinoid with a selective noradrenaline re-uptake inhibitor may offer a beneficial treatment option for neuropathic pain.

1. Introduction

Peripheral nerve injury may lead to neuropathic pain, which is associated with clinical symptoms such as allodynia (nociceptive responses to normally innocuous stimuli), hyperalgesia (augmented pain response to normally painful stimuli) and spontaneous pain. This clinical condition remains poorly treated, even the potent analgesics opioids have limited utility for the treatment of neuropathic pain (Benedetti et al., 1998; Attal, 2012). The tricyclic antidepressant amitriptyline and the anticonvulsant gabapentin are frequently prescribed, but all have incomplete efficacy together with dose-limiting adverse effects. Combining analgesic drugs from different classes is a well-known strategy used for the treatment of clinical pain. The aim was to improve analgesia without enhancing adverse effects or to reduce adverse effects without loss of analgesia (Sarzi-Puttini et al., 2012; Taneja et al., 2012; Attal, 2012).
Although cannabis has been used for recreation and pain management for centuries, it is only recently that it has begun to be used in clinics for the treatment of some central diseases, such as multiple sclerosis, chronic pain, etc. (Grottenhemen and Müller-Vahl, 2012; Lucas, 2012). They produce their effects through cannabinoid-1 (CB1) and cannabinoid-2 (CB2) receptors, but their adverse central effects generally limit their potential clinical application. Cannabinoids and opioids are two different drug classes having many similar pharmacological properties, including analgesia, hypothermia, sedation and reduction in locomotor activity (Fuentes et al., 1999; Pertwee, 2001; Ulugol, 2009). Tolerance and physical dependence may also develop with long-term use of both cannabinoids and opioids (Banashke et al., 2005; Gunduz et al., 2010, 2011b; Ulugol, 2014).

Tapentadol, a novel centrally acting analgesic, combines two different action mechanisms in a single molecule: µ-opioid receptor agonism and noradrenaline re-uptake inhibition (Teschkenke et al., 2006). Tapentadol has been shown to be effective both in nociceptive and chronic pain models; µ-opioid receptor agonism appears to be highly effective against nociceptive pain whereas noradrenaline re-uptake inhibition seems to play the predominant role for the treatment of chronic pain. With the use of tapentadol, not only a synergistic interaction between these components is seen but also an opioid-sparing efficacy is observed ( Harrick and Hernandez, 2012; Taylor et al., 2013; Meske et al., 2014). Here, instead of a µ-opioid receptor agonist, we planned to use WIN 55, 212-2, a non-selective cannabinoid CB1 and CB2 agonist, together with the noradrenaline re-uptake inhibitor, and observe whether an additive or synergistic interaction develops in a neuropathic pain model. As selective noradrenaline re-uptake inhibitors are known to be more effective than selective serotonin re-uptake inhibitors in diminishing pain (Fishbain et al., 2000; Mochizuki, 2004), we preferred to use maprotiline, a selective noradrenaline re-uptake inhibitor. In case of additive or supra-additive results, the combination of a cannabinoid with a selective noradrenaline re-uptake inhibitor might be beneficial for pain relief in humans.

2. Methods

2.1. Animals & Ethics

Male Balb-c mice (Center of the Laboratory Animals, Trakya University), weighing 25–30 g at the beginning of the experiments, were used in the study. Animals were maintained in groups of eight in a quiet room with free access to tap water and food. This study was conducted in agreement with the guidelines of the Ethical Committee of the International Association for the Study of Pain (Zimmermann, 1983), and the scientific protocols were approved by the local ‘Animal Care Ethics Committee’. All efforts were made to minimize animal suffering and the number of animals was kept to a minimum to obtain a dependable experimental data.

2.2. Partial sciatic nerve ligation model of neuropathic pain

2.2.1. Surgery

Partial sciatic nerve ligation (PSNL) model of neuropathic pain, previously described elsewhere, was used in the study. Under ketamine (100 mg/kg) - xylazine (10 mg/kg) anaesthesia, sciatic nerve was isolated and 1/3 to 1/2 of its dorsal portion was tightly tied with 8-0 silk thread (Selitzer et al., 1990; Malmberg and Basbaum, 1998). The muscle and the skin were then sutured separately and mice were allowed to recover in their own cages. In sham-operated animals, the nerve was isolated but not ligated.
2.2.2. Assessment of mechanical allodynia

For the assessment of mechanical allodynia, well-known up-down method was used (Dix, 1965; Chaplin et al., 1994). After choosing nine von Frey filaments (Touch-Test, North Coast Medical, California) with approximately equal logarithmic incremental bending forces (von Frey no.: 1.65, 2.36, 2.44, 2.83, 3.22, 3.61, 4.08, 4.31, 4.56; equivalent to 0.008, 0.02, 0.04, 0.07, 0.16, 0.4, 1, 2, 4 g, respectively), each hair was applied perpendicularly against the plantar surface of the hindpaw until slight bending was seen. Tests were started with the 0.16 g hair and lifting of the paw was regarded as a positive response. In case of a positive response, the next weaker hair was applied; when the mice did not respond, the next stronger hair was tried. These up and down trials were continued until four measurements after the first change in response had been obtained. The pattern of positive and negative responses was converted into a 50% threshold value using the formula given by Dix (1965): 50% threshold = \(10^{x/k}\), where \(x\) is the value of the final von Frey hair used (in log units), \(k\) is the tabular value for the pattern of positive/negative responses, and \(\delta\) is the mean difference in stimuli in log units (0.4). Prior to the assessment of mechanical allodynia, mice were habituated to their wire mesh bottom cages.

2.2.3. Assessment of cold alldynia

Cold allodynia was assessed using a cold/heat plate analgesia meter (Ugo Basile, Comerio, Italy). Mice were placed on a cold plate that is maintained at a temperature of 4.0 ± 0.1 °C, and the latencies to withdrawal of the injured paw were recorded. A cut-off time of 25 s was adjusted to prevent tissue damage. Withdrawal latencies were then converted to the percentage of the maximal possible effect (MPE%) according to the following equation: MPE% = [(postdrug latency − baseline latency)/cut-off time − baseline latency] × 100.

2.2.3. Assessment of locomotor activity

Motor performance was assessed using a rotarod apparatus (Comrat, Ankara, Turkey). Mice were placed on the apparatus and the latencies to fall of the animal were recorded. A cut-off time of 180 s and a speed of 16 rpm were adjusted prior to tests.

2.3. Drugs

The cannabinoid agonist WIN 55,212-2 and the noradrenaline re-uptake inhibitor maprotiline were purchased from Sigma Chemical Co. WIN 55,212-2 was dissolved in 1% ethanol, 1% Tween 80, 20% DMSO and 78% in physiological saline solution, whereas maprotiline was dissolved only in physiological saline. Drugs were administered intraperitoneally (i.p) 30 min before assessments in a volume of 0.1 mL/10 g body weight. In synergy studies, drugs were co-administered in two separate injections. Drug doses and treatment times were selected from previous (Sierralta et al., 1995; Parra et al., 2000; Ulugol et al., 2004, 2006; Banafshe et al., 2005; Ripoll et al., 2006; Gunduz et al., 2011a).

2.4. Experimental design & Statistical analysis

Experiments took place 15 days after sciatic nerve ligation, and each animal was used only once. For standardization, tests were always conducted consecutively and in the same order; mechanical alldynia being the first, cold alldynia the second, and the locomotor activity the last. The influence of one test on the next diminishes when the least stressful test is used at first. Differences in sensitivities and locomotor activity were determined immediately before and 30 min after i.p. injections of WIN 55,212-2 (1, 3, 10 mg/kg) and maprotiline (3, 10, 30 mg/kg). As expected, ED50 values and drug doses in combinations were different in two sensitivity tests. Thus, after determination of dose-response curves, synergy studies were performed using only the cold alldynia test. Test for parallelism of dose-response lines for WIN 55,212-2 and maprotiline was performed according to similar previous studies (Tallarida, 2000, 2001; Luszczki and Florek-Luszczki, 2012).

The interaction of WIN 55,212-2 with maprotiline, with regard to the antinociceptive activity exerted by both drugs in the cold alldynia test, was analysed according to the method of Tallarida (2000). Dose-response curves for WIN 55,212-2 and maprotiline were drawn using 3 dose and 8 animals per dose. ED50 values of each drug alone were then specified by linear regression analysis using the software package Pharm Tools Pro (The McCary Group, Emmaus, PA). ED50 value of maprotiline was found to be higher than the highest non-toxic dose of the drug, which obliged us to use ED50 values of both WIN 55,212-2 and maprotiline in synergy studies. Afterwards, the 1:1, 1:3, and 3:1 fixed ratios of ED50 values of each combination were studied, and these data were assessed as the logarithm of the total dose versus MPE%. The experimentally determined ED50
values of the combination \( Z_{\text{mix}} \) were compared to the theoretically additive ED\(_{10}\) values of the combination \( Z_{\text{add}} \). Drug interactions were considered to be supra-additive \( Z_{\text{mix}} < Z_{\text{add}} \), additive \( Z_{\text{mix}} = Z_{\text{add}} \), or sub-additive \( Z_{\text{mix}} > Z_{\text{add}} \), according to the difference between \( Z_{\text{mix}} \) and \( Z_{\text{add}} \) (Tallarida, 2000, 2001). Statistical analysis of combination data is described previously (Tallarida, 2000, 2001; Codd et al., 2008; Gunduz et al., 2011a). The differences were considered to be significant when \( p \) value was < 0.05. The calculations were made using Pharm Tools Pro (The McCary Group, Emmus, PA).

3. Results

3.1. Effects of tight ligation of the sciatic nerve

Sciatic nerve ligated mice developed pain-related behaviour, known as mechanical and cold alldynia. Prior to surgery, 50% paw withdrawal thresholds for mechanical and MPE% for cold alldynia were 1.91 ± 0.46 and 18.78 ± 1.35, respectively, 15 days after sciatic nerve injury. Withdrawal threshold for mechanical allodynia was 0.79 ± 0.08 \( (p < 0.05) \), and for cold alldynia was 10.46 ± 0.63 \( (p < 0.05) \). In sham-operated mice, 50% paw withdrawal thresholds for mechanical and MPE% for cold alldynia on day 15 (1.03 ± 0.23 and 18.71 ± 1.58, respectively) were similar to those prior to operation (1.34 ± 0.40 and 20.06 ± 1.19, respectively).

3.2. Effects of WIN 55,212-2 and maprotiline on mechanical and cold alldynia

In neuropathic mice, both WIN 55,212-2 and maprotiline exerted dose-dependent anti-allodynic effects in mechanical and cold alldynia tests (Fig. 1A,B). ED\(_{50}\) value of maprotiline was found to be very high; therefore, we used ED\(_{30}\) values of the drugs for the rest of the study. The ED\(_{30}\) values in cold alldynia test were found to be 1.81 ± 0.07 for WIN 55,212-2 and 17.65 ± 8.53 for maprotiline. Test for parallelism revealed that dose-effect curves for WIN 55,212-2 and maprotiline was parallel. The computed \( t \) value in our study (1.832) was lower than the tabular value (4.303), indicating parallelism for these two dose–response lines.

3.3. Isobolographic analysis of interactions between WIN 55,212-2 and maprotiline

Synergy studies between WIN 55,212-2 and maprotiline were performed only in cold alldynia assay. Dose–response curves for WIN 55,212-2, maprotiline and the combination groups are shown in Fig. 2. Isobolographic analysis indicated that interaction of WIN 55,212-2:maprotiline combination in 3:1 and 1:1 ratios of the respective ED\(_{30}\) values of the drugs were supra-additive, and in 1:3 ratio was sub-additive (Fig. 3). Specific doses of each drug, MPE%, and ED\(_{30}\) values are presented in Table 1.

3.4. Effects of WIN 55,212-2 and maprotiline on locomotor activity

No effects on locomotor function were observed in any of the experimental groups, except after treatment with the highest dose of WIN 55,212-2 (***\( p < 0.0001 \)) (Fig. 4).

4. Discussion

As mentioned earlier, clinicians recommend combining analgesic drugs from different pharmacological classes for the treatment of neuropathic pain. Normally, combinations of different mechanisms of action may enable more complete pain relief by targeting pain transmission at different levels or by
Cannabinoids are combined with many drugs from different classes, mostly in experimental studies. Of these, opioid-cannabinoid combination is widely studied. Low dose combinations of these drugs have been shown to be effective in treating both acute and chronic pain (Gichewicz et al., 2004; Smith et al., 2007). A synergistic antinociceptive interaction has been observed between the cannabinoid agonist and the local anaesthetic bupivacaine, the sympatholytic clonidine and the parasympathomimetic neostigmine in the formalin test (Yoon and Choi, 2003; Kang et al., 2007). Moreover, combination of the synthetic cannabinoid WIN 55,212-2 with the non-steroidal anti-inflammatory drug ketorolac resulted with an additive antinociceptive interaction (Ulugu et al., 2006). Recently, synergistic interactions of WIN 55,212-2 both with the anti-epileptic pregabalin and the nociceptin receptor antagonist JTC-801 have been shown using isobolographic analysis (Gunduz et al., 2011a; Luszczki and Florczk, 2012). In this study, we propose another mechanism, noradrenaline re-uptake inhibition, to use together with the cannabinoid for the treatment of neuropathic pain.

Noradrenaline and serotonin act on brain and spinal cord and augment endogenous analgesia mainly by the way of descending inhibitory pathways. Releases of spinal noradrenaline and serotonin are known to play pivotal role in descending inhibition (Millan, 2002). Dual acting antidepressants and specific noradrenaline re-uptake inhibitors are shown to be more effective than selective serotonin re-uptake inhibitors in alleviating pain (Fishman et al., 2000; Mochizuki, 2004). Moreover, the descending inhibitory noradrenergic system is known to enhance opioid-induced antinociception (Meske et al., 2014). Accordingly, combination of µ-opioid receptor agonism and noradrenaline re-uptake inhibition in a single molecule, tapentadol, resulted in an antinociceptive synergy and now being used in acute and chronic pain states (Teschenk and et al., 2006; Harrick and Hernandez, 2012; Taylor et al., 2013). Likewise, cannabinoids also stimulate endogenous noradrenaline release in central, spinal and peripheral sites. Recently, CB1 and CB2 cannabinoid receptor agonists have been suggested to induce antinociception via activation of the endogenous noradrenergic system (Romero et al., 2013). Taking into account, the similarities between opioids and cannabinoids, our findings indicating a partially synergistic interaction between

- WIN 55,212-2
- Maprotiline
- 3:1
- 1:1
- 1:3

Figure 2. Dose-response effects of WIN 55,212-2:maprotiline combination in cold alldynia test in sciatic nerve-injured mice. Each point is the mean ± SEM of 6 animals.

- 3:1 WIN 55,212-2: Maprotiline
- 1:1 WIN 55,212-2: Maprotiline
- 1:3 WIN 55,212-2: Maprotiline

Figure 3. Isobologram illustrating the antinociceptive Interaction between WIN 55,212-2 and maprotiline at 3:1, 1:1, and 1:3 ratio combinations. Filled circles above the straight line represent the theoretical ED50 values of WIN 55,212-2 and maprotiline, while open circles under the straight line represent the experimental ED50 values of the drugs.

Influencing more than one analgesic mechanism. In case of cannabinoids, as these drugs are potent analgesics, combinations will especially be useful to reduce the dose and decrease the incidence of serious central adverse effects of these drugs. Here, we showed for the first time that combination of a cannabinoid with a noradrenaline re-uptake inhibitor might offer a beneficial treatment option for neuropathic pain.
Combining cannabinoids for neuropathic pain

Table 1 Anti-allodynic effects of WIN 55,212-2, maprotiline and WIN 55,212-2:maprotiline combination in neuropathic mice.

<table>
<thead>
<tr>
<th>ED&lt;sub&gt;50&lt;/sub&gt; value ratios (Maprotiline:WIN 55,212-2)</th>
<th>Moprotiline:WIN 55,212-2 drug combination dose (mg/kg i.p.)</th>
<th>% maximal possible effect</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; (SEM)* or Z value (SEI) at 1 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maprotiline only 3:1</td>
<td>1.656 0.057 3.309 0.113 6.616 0.226 13.235 0.453 26.471 0.005</td>
<td>-0.056 1.259 1.079 9.027 12.334 39.917</td>
<td>17.647 (8.522)&lt;sup&gt;a&lt;/sup&gt; 26.550 (13.314)&lt;sup&gt;a&lt;/sup&gt; 13.688 (2.191)</td>
</tr>
<tr>
<td>1:1</td>
<td>0.552 0.057 1.103 0.113 2.206 0.226 4.412 0.453 8.824 0.005</td>
<td>0.176 1.274 1.127 17.342 60.906</td>
<td>4.046 (2.239)&lt;sup&gt;b&lt;/sup&gt; 9.729 (1.460)</td>
</tr>
<tr>
<td>1:3</td>
<td>0.552 0.170 1.103 0.339 2.206 0.679 4.412 1.358 8.824 2.715</td>
<td>0.185 6.802 15.639 36.661 77.904</td>
<td>3.151 (0.701)&lt;sup&gt;c&lt;/sup&gt; 5.769 (0.757)</td>
</tr>
<tr>
<td>WIN 55,212-2 only</td>
<td>1.000 3.000 10.000</td>
<td>7.628 50.040 90.992</td>
<td>1.810 (0.072)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>SEM, standard error of the mean.
<sup>b</sup>Represents ED<sub>50</sub> value of drug alone.
<sup>c</sup>Supra-additive combination.
<sup>d</sup>Sub-additive combination.

Figure 4 Effects of surgery (sham, neuropathic) and drug administrations (WIN 55,212-2, 1-10 mg/kg, maprotiline, 3-30 mg/kg) on spontaneous locomotor activity.

cannabinoids and noradrenaline re-uptake inhibitors was expected.

In our study, combinations of WIN 55,212-2 and maprotiline interacted at only certain ratios of their respective ED<sub>50</sub> values. The interactions of WIN 55,212-2:maprotiline at 1:1 and 3:1 ratios were supra-additive, whereas at 1:3 ratio was sub-additive. These results show that WIN 55,212-2 and maprotiline antinociceptive interaction is synergistic when the cannabinoid component of the combination is increased. Similar combinations appear to be important not only when the drugs interact synergistically but also when the result is additive, because it may reduce specific adverse effects associated with the use of low doses of each drug. The sub-additive interaction at 1:3 ratio, on the other hand, may be counteracted by increasing the cannabinoid component or by testing different doses of these drugs and drug combinations from different classes.
Combining cannabinoids for neuropathic pain


