Synergy between Δ⁹-tetrahydrocannabinol and morphine in the arthritic rat

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Abstract

We have shown in past isobolographic studies that a small amount of Δ⁹-tetrahydrocannabinol (Δ⁹-THC) can enhance morphine antinociception in mice. However, previous studies of the Δ⁹-THC/morphine interaction were performed using normal mice or rats and evaluated acute thermal antinociception. Less is known about cannabinoid and opioid interactions involved in mechanical nociception and in chronic inflammatory pain models, such as Freund’s complete adjuvant-induced arthritic model. One fixed-ratio combination was chosen for testing the interaction between Δ⁹-THC and morphine in the Freund’s adjuvant-induced arthritic model. This combination represented a 1:1 ratio of the drugs and thus consisted of equieffective doses ranging from 0.1 to 5 mg/kg Δ⁹-THC and from 0.1 to 5 mg/kg morphine. The combination ED₅₀ value for the fixed ratios (total dose) in relation to the ED₅₀ value of the drugs alone was determined. The isobolographic analysis indicated a synergistic interaction between Δ⁹-THC and morphine in both the non-arthritic and the arthritic rats. Since Freund’s adjuvant-induced alteration in endogenous opioid tone has been previously shown, our data indicate that such changes did not preclude the use of Δ⁹-THC and morphine in combination. As with acute preclinical pain models in which the Δ⁹-THC/morphine combination results in less tolerance development, the implication of the study for chronic pain conditions is discussed.

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Keywords: Isobologram; Synergy; Arthritis; Δ⁹-THC; Morphine

1. Introduction

Cannabinoids and opioids share several pharmacological effects, including hypothermia, sedation, analgesia, and the inhibition of motor activity (Bloom and Dewey 1978; Mannarino et al., 1999) through actions at cannabinoid and opioid receptors, members of the G-protein-coupled receptor family. Activation of G-protein-coupled receptors produces intracellular events such as inhibition of adenylate cyclase activity (Sharma et al., 1975; Howlett and Fleming, 1984), decreased calcium influx, and increased potassium efflux (Morita and North, 1982; Hescheler et al., 1987; Felder et al., 1992). Cannabinoid CB₁ receptors and mu-opioid receptors have been reported to co-localize in brain areas involved in nociceptive responses such as the periaqueductal grey, amygdala, and thalamus (Mansour et al., 1988; Martin et al., 1999). These structures are part of a descending pain control circuit that mediates pain suppressive actions of both opioids and cannabinoids (Basbaum and Fields, 1984; Meng et al., 1998). In the spinal cord, opioid and cannabinoid receptors are co-localized in areas of the dorsal horn where they are involved in nociceptive control (Salio et al., 2001).

Endogenous opioids have been shown to mediate cannabinoid-induced antinociception. Δ⁹-THC-induced antinociception was found to be modulated by mu-opioid receptors supraspinally, while kappa-opioid receptors were involved in spinal antinociception (Smith et al., 1994; Reche et al., 1996). Spinally administered Δ⁹-THC releases dynorphin A in the spinal cord of the rat (Mason et al., 1999). Δ⁹-THC and morphine administration by any combination of routes significantly enhances the potency of morphine in mice (Welch and Stevens, 1992; Smith et al., 1998a). A later study (Cichewicz et al., 1999) confirmed that a non-antinociceptive oral dose of Δ⁹-THC enhances the potency of an acute oral dose of morphine, as well as other opioid analgesics. Furthermore, a full isobolographic analysis of the interaction between oral Δ⁹-THC and morphine or codeine provided evidence of synergy between Δ⁹-THC and these opioids (Cichewicz and McCarthy, 2003).
Previous studies of the \(\Delta^9\)-THC/morphine interaction were performed using normal mice or rats and evaluated acute thermal antinociception. Less is known about cannabinoid and opioid interactions involved in mechanical nociception and in chronic inflammatory pain models, such as Freund’s complete adjuvant-induced arthritic model. Freund’s adjuvant treatment produces chronic inflammation, edema, and hyperalgesia in rats (Millan et al., 1986a). Sofia et al. (1973) demonstrated that \(\Delta^9\)-THC is effective in the paw-pressure test for mechanical nociception in rats. In Freund’s adjuvant-induced arthritic rats, \(\Delta^9\)-THC-elicited antinociceptive efficacy was no different from that in normal rats (Smith et al., 1998b). However, \(\Delta^9\)-THC modulation of dynorphin A was found to differ in normal versus arthritic rats. While \(\Delta^9\)-THC was shown to trigger an increase in release of dynorphin A in normal rats, arthritic rats had high levels of dynorphin A and \(\Delta^9\)-THC normalized dynorphin A levels to that of normal animals (Cox and Welch, 2004). Since endogenous opioid release has been shown to play a major role in the enhancement of morphine by \(\Delta^9\)-THC in acute pain models (Pugh et al., 1996), our study was designed to determine if this enhancement was observed in arthritic and normal rats using mechanical stimuli.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN), which weighed 350 to 375 g were housed in an animal care facility maintained at 22 ± 2 °C on a 12-h light/dark cycle with free access to food and water. All experiments were conducted according to guidelines established by the Institutional Animal Care and Use Committee of Virginia Commonwealth University and adhere to the guidelines of the Committee for Research and Ethical Issues of IASP.

2.2. Freund’s adjuvant-induced arthritis treatment

A volume of 0.1 ml of vehicle (mineral oil) or Freund’s complete adjuvant (heat-killed Mycobacterium butyricum; 5 mg/ml) was injected intradermally into the base of the tail. Animals remained in their cages for 12 days and were acclimated daily to paw-pressure testing until day 19, on which they were tested. Acclimation consisted of a brief pressure stimulus, less than that necessary to elicit paw withdrawal, to the hind paw of the rat using the paw-pressure apparatus. Inflammation proceeds into a generalized polyarthritis within 19 days. Paw-pressure baseline measurements on day 19 indicated that arthritic rats are more sensitive to mechanical nociception than non-arthritic rats.

2.3. Paw-pressure test

The paw-pressure test consisted of gently holding the body of the rat while the hind paw was exposed to increasing mechanical pressure. The Analgesy-Meter (Ugo-Basile, Varese, Italy) is designed to exert a force on the paw that increases at a constant rate, similar to the Randall and Sellito (1957) test of mechanical nociception. Force was applied to the hind paw that was placed under a small plinth under a cone-shaped plunger with a rounded tip. The operator depressed a pedal-switch to start the mechanism that exerted force. The force in grams at which the rat withdrew its paw was defined as the paw-pressure threshold. The baseline paw-pressure was measured before injecting vehicle or drug. Non-arthritic rats that had a baseline paw-pressure greater than 100 g (average = 177 ± 6.42 g) were used in further testing. Arthritic rats that had a baseline paw-pressure less than 100 g (average = 71 ± 3.05 g) were used in further experimentation. The upper limit of 500 g was imposed for the experiments to allow the foot to not become immobilized due to undue pressure.

2.4. Drug administration protocol

For the generation of dose–response curves using the paw-pressure test of antinociception in arthritic and non-arthritic rats, morphine and \(\Delta^9\)-THC were administered i.p. The dose–response curves for morphine and \(\Delta^9\)-THC alone were determined. Morphine was prepared in distilled water and \(\Delta^9\)-THC was prepared in a solution of emulphor, ethanol, and saline at a 1:1:18 ratio. Morphine (0.5, 1, 2, and 4 mg/kg) or distilled water vehicle was administered 30 min prior to antinociceptive testing. \(\Delta^9\)-THC (0.5, 1, 2 and 4 mg/kg) or 1:1:18 vehicle was administered 30 min prior to antinociceptive testing. The peak times for antinociception produced by \(\Delta^9\)-THC and morphine have been previously determined to be 30 min post administration (Cox and Welch, 2004).

2.5. Percent maximum possible effect determination

The average paw-pressure threshold in grams was determined before drug administration (baseline) and the selected times (test) following drug administration. The maximum possible effect (%MPE) was determined for each rat according to the following formula using a 500 g maximum pressure: %MPE = \(\frac{\text{test (g)} - \text{baseline (g)}}{500 \text{ g} - \text{baseline (g)}} \times 100\). Dose–response curves were generated using three or four doses of test drug. ED50 values and 95% confidence limits were determined using the methods of Tallarida and Murray (1987). Injection of distilled water (i.p., 0.1 cc/100 g body weight) resulted in a %MPE less than 5 ± 1%. Injection of 1:1:18 emulphor:ethanol:saline vehicle resulted in a %MPE of 8 ± 2%.

2.6. Isobolographic analysis

The use of the isobologram has been reviewed extensively in the context of drug combination studies (Wessinger, 1986; Tallarida et al., 1989; Tallarida, 2001). The method used in the present studies is similar to that reported by Kimmel et al. (1997). Dose–response curves were generated for each drug alone, and ED50 values (dose which yields 50% effect) and standard error (S.E.M.) were computed using unweighted least-squares linear regression as modified from procedures 5 and 8 described by Tallarida and Murray (1987). The ED50 values of
the drugs alone are then plotted and a theoretical additive line is constructed on an isobologram. Experimental values from the fixed-ratio design studies were also analyzed using linear regression and an ED50 value for each combination was determined and plotted on the isobologram for comparison to the theoretical additive value. This theoretical value, termed $Z_{\text{add}}$, is calculated using the formula $Z_{\text{add}} = fz_1 + gz_2$ (Tallarida et al., 1997, Eq. (3)), where $f + g = 1$ (the proportions of each drug) and $z_1$ and $z_2$ represent the ED50 values for each drug alone. The standard error for $Z_{\text{add}}$ is determined from the formula: $\text{S.E.M.}(Z_{\text{add}}) = \sqrt{f^2 \text{S.E.M.}(z_1)^2 + g^2 \text{S.E.M.}(z_2)^2}$ (Tallarida et al., 1997, Eq. (4)). The Student’s $t$-test was used to determine statistical significance of the difference between the logarithmic equivalents of the ED50 values (since a requirement of the $t$-test is the use of values that are normally distributed). A more detailed explanation of the calculations used for the $t$-test can be found in the literature (Tallarida, 2001). A $P$ value less than 0.05 indicated that the drugs produced a synergistic effect.

### Table 1

<table>
<thead>
<tr>
<th>$\Delta^9$-THC (micrograms/kg)</th>
<th>Morphine (micrograms/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-arthritic rats</td>
<td>Non-arthritic rats</td>
</tr>
<tr>
<td>$z_1 = 2100 \pm 300$</td>
<td>$z_2 = 2400 \pm 400$</td>
</tr>
<tr>
<td>$\log z_1 = 3.222 \pm (2.48)$</td>
<td>$\log z_2 = 3.38 \pm (2.60)$</td>
</tr>
</tbody>
</table>

Arthritic rats

| $z_1 = 2500 \pm 500$          | $z_2 = 2200 \pm 400$      |
| $\log z_1 = 3.40 \pm (2.70)$ | $\log z_2 = 3.34 \pm (2.60)$ |

Amounts are total (in micrograms/kg for ease of log calculations).

### 2.7. Statistical analysis

The average paw-pressure threshold in grams was determined before drug administration (baseline) and the selected times (test) following drug administration. The maximum possible effect (%MPE) was determined for each rat according to the following formula using a 500 g maximum pressure: $\% \text{MPE} = \frac{\text{test (g)} - \text{baseline (g)}}{500 \text{ g} - \text{baseline (g)}} \times 100$.

Dose–response curves were generated using at least three doses of test drug. ED50 value and 95% confidence limits were determined using the methods of Tallarida and Murray (1987).

### 2.8. Drugs

Freund’s complete adjuvant was prepared in mineral oil supplied by Sigma Chemical Co. (St. Louis, MO). Freund’s complete adjuvant contains heat-killed *M. butyricum* and is supplied by Difco Laboratories (Detroit, MI). Morphine and $\Delta^9$-THC were obtained from the National Institute on Drug Abuse (Rockville, MD).
3. Results

3.1. Dose–response analysis of drugs alone

Figs. 1 and 2 show the dose–response curves for the antinoceptive effects of morphine and Δ⁹-THC respectively alone in rats. ED₅₀ values (z₁, z₂) and S.E.M. for each drug, as well as logarithmic equivalent doses, are presented in Table 1. Each of the ED₅₀ values is in accordance with earlier studies (Cox and Welch, 2004). These values represent the equieffective doses of the drugs in these studies. The ED₅₀ value for morphine with 95% confidence limits was 2.4 mg/kg (2.2–2.8) in normal rats. In arthritic rats, the ED₅₀ value for morphine with 95% confidence limits was 2.2 mg/kg (1.9–2.4). The ED₅₀ value for Δ⁹-THC with 95% confidence limits was 2.1 mg/kg (1.8–2.5) in normal rats. In arthritic rats, the ED₅₀ value for Δ⁹-THC with 95% confidence limits was 2.5 mg/kg (2.2–3.0). Thus, both drugs were equipotent and equiefficacious in the non-arthritic and the arthritic rats.

3.2. Isobolographic analysis of Δ⁹-THC/morphine interactions

One fixed-ratio combination was chosen for testing the interaction between Δ⁹-THC and morphine. This combination represented a 1:1 ratio of z₁:z₂ and thus consisted of equieffective doses ranging from 0.1 to 5 mg/kg Δ⁹-THC and from 0.1 to 5 mg/kg morphine. We have shown in past isobolographic studies that a small amount of Δ⁹-THC can enhance morphine antinociception in mice (Cichewicz and McCarthy, 2003). Figs. 3 and 4 show the plots of the combination ED₅₀ values for the fixed ratios (total dose) in relation to the ED₅₀ values of the drugs alone. The theoretical additive point for each drug combination is indicated on the graph by A, while the experimental points for each drug combination are indicated on the graph by B. The isobologram indicates that a synergistic interaction occurs between Δ⁹-THC and morphine in both the non-arthritic and the arthritic rats since the experimental points lie significantly below the line of additivity. This graphical display of synergism is confirmed mathematically by comparison of the experimental values to the theoretical values using the Student’s t-test. Table 2 lists the experimental and additive ED₅₀ values and S.E.M. as well as their logarithmic equivalents. For the ratio tested, the experimental value is less than the calculated additive value, and the difference is statistically significant (P<0.05); thus, the combination of Δ⁹-THC and morphine shows synergism in both non-arthritic and arthritic rats.

4. Discussion

The first goal of the study was to determine if Δ⁹-THC enhanced morphine-induced antinociception in the paw-pressure test in both normal and Freund’s complete adjuvant-induced arthritic rats. If an enhancement of antinociception was observed, we wanted to determine if the two drugs had a synergistic interaction. To determine if Δ⁹-THC would enhance morphine-induced antinociception, a combination of a fixed low dose of Δ⁹-THC (0.5 mg/kg) with low doses of morphine was tested in the paw-pressure test. Our results indicated that in both non-arthritic and arthritic rats, the morphine-dose–response curve was shifted to the left (data not shown). This preliminary study led us to examine whether Δ⁹-THC would have an additive interaction with morphine or a synergistic relationship using isobolographic analyses. An additive interaction describes the simple addition of the expected effects of each dose of drug alone, while synergism represents the combined effect of the drugs, which greatly exceeds that expected by simple addition. We found that Δ⁹-THC and morphine, in a simple fixed dose ratio of 1:1 (z₁:z₂), displayed a synergistic interaction.

The synergistic interaction that we demonstrated was predicted by previous findings. Early studies by Ghosh and Bhattacharya (1979) in rats demonstrated the enhancement of morphine injected i.p. by an extract of Cannabis indica by the same route. Several years later, the potency of codeine and morphine given orally was found to be enhanced by orally administered Δ⁹-THC and Δ⁸-THC in mice (Mechoulam et al., 1984). More recently, low doses of Δ⁹-THC were found to...
significantly enhance morphine in the tail-flick test in the mouse when both were administered intrathecally (Welch and Stevens, 1992), and i.e.v. administration of both drugs resulted in an enhancement of morphine-induced antinociception (Welch et al., 1995). It was further established that Δ⁹-THC enhanced the potency of morphine in any combination of s.c. and p.o. in mice in the tail-flick test, and that both given s.c. enhanced the potency of morphine in mice in paw withdrawal to radiant heat (Smith et al., 1998a). The enhancement of both morphine and codeine by Δ⁹-THC, administrated p.o., was found to be not just additive but synergistic interactions, determined by isobolographic analysis (Cichewicz and McCarthy, 2003).

The mechanisms of the enhancement of morphine by Δ⁹-THC have also been examined. Naloxone (s.c.) was shown to block the antinociceptive effects of the combination of i.t. Δ⁹-THC and morphine, while having no effect on Δ⁹-THC alone (Welch and Stevens, 1992). Antinociception produced by intrathecal administration of Δ⁹-THC and morphine was attenuated by the kappa-opioid receptor antagonist, norBNI, and the delta-opioid receptor antagonist, naltrindole. Administration of a cocktail of enzyme inhibitors used to prevent the metabolism of dynorphin A (1–8) into leucine-enkephalin also attenuated the enhancement of morphine-induced antinociception by Δ⁹-THC (Pugh et al., 1996). Taken together these data imply a role of delta- and kappa- opioids in the enhancement of morphine antinociception by Δ⁹-THC in the spinal cord. The hypothesis put forth is that the delta- and kappa-opioid interaction involves the Δ⁹-THC-induced release of dynorphin and its metabolism to leu-enkephalin, and that mu-, kappa-, and delta-opioid receptors are required for a maximum antinociceptive effect of the opioid and cannabinoid combination.

These previous findings led us to hypothesize that Δ⁹-THC would enhance morphine, both administered parenterally, in the paw-pressure test in the rat. However, we also wanted to examine this interaction in a Freund’s adjuvant-induced arthritic rat. The endogenous opioid system has been shown to be altered in the arthritic rat. Rats with chronic pain of polyarthritis exhibit alterations in the activity of the central nervous system (CNS) and hypophyseal pools of β-endorphin, met-enkephalin, and dynorphin (Millan et al., 1985, 1986a,b). Increases in tissue levels of dynorphin and met-enkephalin and mRNA encoding their precursors in the dorsal horn of the spinal cord have been reported. While tissue and mRNA levels correlate with an increase in spinal release of dynorphin A, a decrease in met-enk release was observed (Millan et al., 1986a; Pohl et al., 1997; Ballet et al., 2000). We have demonstrated that while Δ⁹-THC i.p. triggered a spinal release of dynorphin A in a normal rat, consistent with Mason et al. (1999), Δ⁹-THC administration resulted in a lesser amount of dynorphin A in the spinal cord of arthritic rats than that after vehicle treatment (Cox and Welch, 2004). At the same time, however, Δ⁹-THC-induced antinociception was found to be no different in non-arthritic versus arthritic rats (Smith et al., 1998b; Cox and Welch, 2004). In a similar manner, the degree of synergy of the dose combination of Δ⁹-THC and morphine in this study was found to be the same in both non-arthritic and arthritic rats. Given that Δ⁹-THC in arthritic rats decreases dynorphin A levels, but releases dynorphin A in non-arthritic rats (Cox and Welch, 2004), the mechanism by which Δ⁹-THC and morphine act synergistically in the arthritic rat remains unclear.

However, several important and clinically relevant points result from this study: 1) Δ⁹-THC is a potent antinociceptive agent in chronic Freund’s complete adjuvant-induced pain. Δ⁹-THC is equipotent and equiefficacious to morphine in this test system. In other models of acute pain in humans or animals, Δ⁹-THC lacks the potency and efficacy of morphine (Buxbaum, 1972; Bloom et al., 1977; Smith et al., 1998a); 2) The Δ⁹-THC/morphine combination results in synergistic interaction in both normal and arthritic rats. Thus, the Freund’s adjuvant-induced alteration in endogenous opioid tone does not preclude the use of Δ⁹-THC and morphine in combination; 3) as with acute preclinical pain models in which the Δ⁹-THC/morphine combination results in less tolerance development, it is likely that such might be the case in long-term treatment of chronic pain. In summary, the synergistic interaction of Δ⁹-THC and morphine in arthritic rats, as well as the potency of Δ⁹-THC in the CFA chronic pain condition, points to the possibility of the use of the combination, or to the use of a Δ⁹-THC analog, to treat chronic pain conditions for which opioids alone are ineffective.

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References


