Antinociceptive Synergy Between Delta 9-Tetrahydrocannabinol and Opioids After Oral Administration

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Abbreviations:
$\Delta^9$-THC delta 9-tetrahydrocannabinol  $ED_{50}$ effective dose 50%
p.o. per os (oral) S.E. standard error
% MPE percent maximum possible effect CB1 cannabinoid receptor 1

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Abstract

The analgesic effects of opioids such as morphine and codeine in mice are enhanced by oral administration of the cannabinoid delta 9-tetrahydrocannabinol (Δ⁹-THC). However, isobolographic analysis has never been done to confirm a synergy between Δ⁹-THC and morphine or codeine via oral routes of administration. In order to determine the nature of the interaction between these drugs for pain relief and extend previous experimental results, we performed an isobolographic analysis to evaluate for additivity or synergy in the tail flick test. Fixed-ratio combinations of Δ⁹-THC with either morphine or codeine were tested for antinociceptive effects. The experimentally derived ED₅₀ for each combination was compared to the theoretical additive ED₅₀ using an isobolographic analysis. All of the fixed-ratio combinations tested produced greater antinociception (synergy) than predicted from simple additivity. These findings suggest that the use of a low dose combination of analgesics is a valid and effective approach for the treatment of pain and necessitates further study.
Cannabinoids and opioids have been shown to possess several similar pharmacological effects, including analgesia, sedation, hypothermia and inhibition of motor activity (Holtzman et al., 1969; Bloom and Dewey, 1978; Bhargava, 1980). Studies using the synthetic cannabinoid CP55,940 have shown that cannabinoids exhibit a similar binding distribution in brain to that of morphine (Kuhar et al., 1973; Mailleux and Vanderhaeghen, 1992). In addition, these two classes of drugs produced similar effects on calcium levels and cyclic AMP accumulation through G-protein-mediated pathways (Bidaut-Russell and Howlett, 1988; Pugh et al., 1994).

Since the discovery that opioids and cannabinoids produce not only several similar biochemical effects but pharmacological effects as well, the interaction between these two classes of drugs has been studied extensively (for review, see Manzaneres et al., 1999). Cannabinoids have been shown to produce analgesia through interaction with kappa opioid receptors in the spinal cord (Smith et al., 1994; Pugh et al., 1996; Reche et al, 1996). It was further determined that cannabinoids evoked the release of endogenous opioids that stimulate delta and kappa opioid receptors to produce antinociception (Welch, 1993; Pugh et al., 1996). The discovery of a bi-directional cross-tolerance of cannabinoids to kappa opioid agonists (Smith et al., 1994) and to morphine (Thorat and Bhargava, 1994) in analgesic tests provided further evidence that the cannabinoid and opioid antinociceptive pathways were linked.

A synergism between the cannabinoid delta 9-tetrahydrocannabinol (Δ9-THC) and morphine has already been suggested in the spinal cords of mice (Welch and Stevens, 1992). Reche et al. (1996) demonstrated a greater-than-additive interaction between Δ9-THC and morphine administered i.v., since inactive doses of the drugs in combination produced a potent analgesic effect. This combination of drugs produced effects through both a mu opioid receptor- and CB1 cannabinoid receptor-mediated pathway, since the potentiation was completely blocked
by SR141716A, the selective CB1 antagonist, and by β-funaltrexamine, a selective mu antagonist (Reche et al., 1996). Recent studies have shown that Δ⁹-THC significantly enhanced the potency of morphine and codeine in the tail flick test for antinociception by any two routes (i.t., i.c.v., s.c., p.o.) of administration (Smith et al., 1998; Cichewicz et al., 1999). Research has also shown that the antinociceptive effects, but not the hypothermic or cataleptic effects, of cannabinoids are enhanced by the presence of morphine (Smith et al., 1994; Welch and Eades, 1999).

The study of the interaction between cannabinoids and opioids in the prevention of pain is quite significant, when considering chronic pain and the possibility of smaller doses yielding fewer side effects and less addiction potential. In order to understand further the mechanisms underlying Δ⁹-THC/morphine or Δ⁹-THC/codeine interactions, the effects of these combinations were determined by the use of isobolographic analysis to evaluate drug synergy. Many previous studies have used this technique to investigate interactions between classes of drugs (Kimmel et al., 1997; Roth and Rowland, 1999; Kolesnikov et al., 2000) and specifically, opioids (Roerig et al., 1991; Miaskowski et al., 1992; Raffa et al, 1993). Detailed explanations of the statistics and design of such experiments have been published (Tallarida et al., 1997; Tallarida, 2001). We have been able to effectively adapt this type of analysis for antinociceptive testing, in order to determine if the effect of the combination is equal to or greater than the expected additive effect of the individual drugs at the same doses. Examination of oral combinations of Δ⁹-THC and morphine or codeine in mice have never been subjected to isobolographic analysis in this fashion. In addition, most of our previous studies have utilized Δ⁹-THC p.o. at a low dose of 20 mg/kg to enhance the antinociception produced by opioids, but until now, examination of lower Δ⁹-THC doses had not been performed.
The experiments presented in this paper examined various combinations of $\Delta^9$-THC and two opioids, morphine and codeine, via an oral route of administration in mice. Data was collected using the tail flick test for antinociception and then plotted as an isobologram to determine the nature of the drug interactions. In contrast to our previous studies, these experiments utilize our first bi-directional design, in which doses of both drugs in the combination are varied. Based on previous findings, we hypothesized that $\Delta^9$-THC and opioids would produce a greater-than-additive effect on antinociception.
Materials and Methods

Animals.  Male ICR mice (Harlan Laboratories, Indianapolis, IN) weighing 25 to 30 g were housed 3 per cage in an animal care facility maintained at 22 ± 2°C on a 12-h light/dark cycle. Food and water were available ad libitum. The mice were brought to the test room 24 h prior to the test day to allow acclimation and recovery from transport and handling. All experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

Drugs.  Morphine sulfate, codeine phosphate and delta 9-THC were obtained from the National Institute on Drug Abuse (Bethesda, MD). Morphine and codeine were dissolved in distilled water, while Δ⁹-THC was prepared in ethanol, Alkamuls-EL620™ (Rhodia, Cranbury, NJ), and saline in a 1:1:18 ratio. All drugs were administered by oral gavage.

Drug administration protocol. For dose-response curves for drugs alone, all drugs were administered p.o. 30 min prior to antinociceptive testing. A minimum of four doses was tested to generate a comprehensive dose-response curve for each drug. For dose-response curves of the drugs in combination, Δ⁹-THC was administered p.o. 15 min prior to morphine p.o. or 30 min prior to codeine p.o. These time points were previously established to result in maximal enhancement of the opioid antinociceptive effects by Δ⁹-THC (Cichewicz et al., 1999). The mice were then tested 30 min later for antinociception.
Tail flick test for antinociception. The tail flick heat latency test for antinociception was designed by D’Amour and Smith (1941). Baseline tail flick latencies were determined prior to drug administration on the test day and were between 2 and 4 sec. During drug testing, a cutoff time of 10 sec was employed to prevent damage to the tail. Antinociception was quantified using the percentage of maximum possible effect (% MPE) calculated as developed by Harris and Pierson (1964) as follows: \( \% \text{ MPE} = \frac{(\text{test} - \text{baseline})}{(10 - \text{baseline})} \times 100 \). Each test group contained 6 mice, and a mean % MPE value was determined for each group.

Isobolographic analysis. The use of the isobologram has been reviewed extensively in the context of drug combination studies (Wessinger, 1986; 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 ED\(_{50}\) values (dose which yields 50% effect) and standard error (S.E.) were computed using unweighted least-squares linear regression as modified from procedures 5 and 8 described by Tallarida and Murray (1987). The ED\(_{50}\) 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 ED\(_{50}\) 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 ED\(_{50}\) values for each drug alone. The standard error for Z\(_{\text{add}}\) is determined from the formula \( \text{SE}(Z_{\text{add}}) = \left[ f^2 \{ \text{SE}(z_1) \}^2 + g^2 \{ \text{SE}(z_2) \}^2 \right]^{1/2} \) (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 ED\(_{50}\) 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, 2000). A $p$ value less than 0.05 indicated that the drugs produced a synergistic effect.
Results

Dose-response analysis of drugs alone. Fig. 1 shows the dose-response curves for the antinociceptive effects of morphine, codeine and $\Delta^9$-THC alone in mice. Each drug was administered p.o. ED$_{50}$ values ($z_1$, $z_2$, $z_3$) and S.E. for each drug, as well as logarithmic equivalent doses, are presented in Table 1. Each of the ED$_{50}$ values is in accordance with earlier studies (Cichewicz et al., 1999). These values represent the equieffective doses of the drugs in these studies.

Isobolgraphic analysis of $\Delta^9$-THC/morphine interactions. Two fixed-ratio combinations were chosen for testing the interaction between $\Delta^9$-THC and morphine. The first combination represented a ratio of $z_1:z_2$ and thus consisted of equieffective doses ranging from 5-35 mg/kg $\Delta^9$-THC and from 1-10 mg/kg morphine. The second combination represented a ratio of $0.1z_1:0.9z_2$, since we have shown in past studies that a small amount of $\Delta^9$-THC can enhance morphine antinociception (Cichewicz et al., 1999). The doses tested for this second combination ranged from 1-27 mg/kg $\Delta^9$-THC and from 2-67 mg/kg morphine.

An isobologram was constructed to determine whether $\Delta^9$-THC and morphine produce antinociception in a synergistic manner. Fig. 2 shows the plots of the combination ED$_{50}$ values for both fixed ratios (total dose) in relation to the ED$_{50}$ values of the drugs alone. The theoretical additive points for each drug combination are indicated on the graph by A and B, while the experimental points for each drug combination are indicated on the graph by C and D. The isobologram indicates that a synergistic interaction occurs between $\Delta^9$-THC and morphine 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 ED$_{50}$ values and S.E. as well as their logarithmic equivalents. For each ratio tested, the experimental value is less than the calculated additive value, and the difference is statistically significant ($p < 0.05$); thus, each combination of $\Delta^9$-THC and morphine shows synergism.

**Isobolographic analysis of $\Delta^9$-THC/codeine interactions.** Two fixed-ratio combinations were also chosen for testing the interaction between $\Delta^9$-THC and codeine. The first combination represented a ratio of $z_1:z_3$ and thus consisted of equieffective doses ranging from 5-30 mg/kg $\Delta^9$-THC and from 4-27 mg/kg codeine. The second combination represented a ratio of $0.2z_1:0.8z_2$, since we have shown in past studies that a small amount of $\Delta^9$-THC can also enhance codeine antinociception (Cichewicz et al., 1999). The doses tested for this second combination ranged from 5-18 mg/kg $\Delta^9$-THC and from 17-63 mg/kg codeine.

An isobologram was constructed in a similar fashion as above to determine whether $\Delta^9$-THC and codeine produce antinociception in a synergistic manner. Fig. 3 shows the plots of the combination ED$_{50}$ values for both fixed ratios (total dose) in relation to the ED$_{50}$ values of the drugs alone. The theoretical additive points for each drug combination are indicated on the graph by A and B, while the experimental points for each drug combination are indicated on the graph by C and D. The isobologram indicates that a synergistic interaction occurs between $\Delta^9$-THC and codeine 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 3 lists the
experimental and additive ED$_{50}$ values and S.E. as well as their logarithmic equivalents. For each ratio tested, the experimental value is less than the calculated additive value, and the difference is statistically significant ($p < 0.05$); thus, each combination of Δ$^9$-THC and codeine shows synergism.
Discussion

Morphine and codeine are two commonly used opioids for the control of pain, whether alone or in combination with an adjunct drug. Both drugs produce full efficacy in the tail-flick test for antinociception. Unfortunately, long-term use of these drugs results in the development of tolerance and physical dependence, reducing their analgesic effects and necessitating high, potentially harmful doses. Thus, combination therapy seems to be a promising field in which two low doses of drugs can be administered simultaneously to produce high analgesic effects without adverse side effects. We have previously shown that the acute antinociceptive effects of morphine and codeine are greatly enhanced by a low 20 mg/kg dose of Δ⁹-THC (Smith et al., 1998; Cichewicz et al, 1999). However, without examination of the interaction between these drugs, a single-dose study does not provide a complete picture of the magnitude of this enhancement. Thus, in the present paper, we have looked at various dose combinations to extend previous experimental findings in single-dose studies and further characterize this interaction between cannabinoid and opioid analgesic pathways. These experiments represent the first in-depth study of oral combinations of Δ⁹-THC and opioids for synergy and are a significant advance in the investigation of cannabinoid/opioid interactions.

In performing an isobolographic analysis, one must consider the benefits of a synergistic relationship over an additive interaction. Additivity represents the simple addition of the expected effects of each dose of drug alone, whereas synergy describes a situation in which the combined effect greatly exceeds that expected with simple addition. Clearly, synergistic drug interactions would be much more significant, indicating that low doses of drugs together could produce effects of high magnitude. Our previous findings with Δ⁹-THC and morphine or
codeine suggested a greater-than-additive effect, which we were able to confirm with isobolographic analyses in the present studies.

The results presented in this paper indicate that Δ⁹-THC and these two opioids do produce synergistic antinociception after oral administration. Thus, the combinations produced analgesic effects greater than those predicted by a simple addition of their separate effects. It is important to note that a 20 mg/kg p.o. dose of Δ⁹-THC alone produces less than 10% MPE in the tail flick test (Cichewicz et al., 1999). This observation only strengthens the argument that Δ⁹-THC acts synergistically with the opioids, since this inactive dose is able to greatly enhance the analgesic effects of morphine and codeine. The clinical benefits of such an enhancement can be easily imagined, as it would allow for the prescription of much lower drug doses which would still yield high analgesic effect, yet induce fewer side effects (e.g. morphine-induced respiratory depression) that would normally accompany high drug doses.

Many previous reports have described synergistic relationships between drugs that target different neurotransmitter systems (Wellman et al., 1995; Roth and Rowland, 1999; Kolesnikov et al., 2000). However, in these studies, we find that synergy can exist between two classes of drugs which are known to have similar effects at the receptor level and at the second messenger level. Morphine and codeine produce analgesia through G protein-coupled opioid receptors in the brain and spinal cord (Neil, 1984; Pasternak, 1993; Cichewicz et al., 1999). Δ⁹-THC can also act through opioid receptors to produce analgesia, by releasing or increasing the transcription of endogenous opioids (Smith et al., 1994; Pugh et al., 1996; Corchero et al., 1997), and in fact, synergistic interactions have been suggested with endogenous opioids and cannabinoids (Welch and Eades, 1999). In addition, both Δ⁹-THC and morphine have been shown to decrease the levels of cAMP and intracellular free calcium in the brain (Bidaut-Russell and Howlett, 1988;
Pugh et al., 1994). Reche and colleagues (1996) speculated that cannabinoid and mu-opioid receptors activate similar descending inhibitory pathways regulating the release of nociceptive neurotransmitters. Thus, the synergy we observe with Δ⁹-THC and morphine or codeine most likely results from enhanced activation of the opioid receptor cascade. This is in agreement with data from our previous work, demonstrating that the enhancement of opioids by Δ⁹-THC is blocked by naloxone (Cichewicz et al., 1999). Miaskowski et al. (1992) and others further suggest that all three types of opioid receptors may interact to produce antinociceptive synergy (Vaught et al., 1982; Sutters et al., 1990).

The times between administrations of the drugs may be critical to the enhancement effect. After several earlier studies carried out with various time points between the administrations of Δ⁹-THC and morphine or codeine, we concluded that the optimal pretreatment time for Δ⁹-THC p.o. to enhance the analgesic effect of these opioids in the tail flick test was 15-30 min (Smith et al., 1998; Cichewicz et al., 1999). We have been unable to enhance Δ⁹-THC analgesia with a pretreatment of morphine or codeine, and therefore, while our synergistic interactions appear to be one-way at this point, it is possible that experimenting with other time points between the two drugs may yield a bi-directional synergy.

We have previously hypothesized that the antinociceptive effects of morphine, which are primarily mediated though mu opioid receptors, are enhanced by Δ⁹-THC through the activation of kappa and delta receptors (Pugh et al., 1996). It is also possible that this enhancement requires a physical or functional coupling between mu and delta, or mu and kappa, opioid receptors. Many studies illustrate intimate associations between mu and delta opioid receptors. For example, knock-out studies show that not only delta but mu receptors are required for delta ligand-mediated antinociception (Sora et al., 1997). Others suggest that formation of mu/delta
heterodimers may explain the enhancement of mu receptor-mediated analgesia by delta-specific ligands (Traynor and Elliot, 1993; Gomes et al., 2000). These observations followed previous work demonstrating an allosteric coupling between morphine and enkephalin receptors \textit{in vitro} (Rothman and Westfall, 1982). Thus, the enhancement of morphine analgesia by $\Delta^9$-THC could be occurring not only through the release of endogenous opioids which might interact with proximal opioid receptors, but also through a direct stimulation of receptor coupling. Co-immunoprecipitation studies performed in combination-treated mice could be proposed to examine the possibility of receptor heterodimers.

In summary, we have observed that $\Delta^9$-THC enhances the antinociceptive effects of morphine and codeine in a synergistic fashion. This is the first report of a true synergistic interaction between oral $\Delta^9$-THC and morphine or codeine, since previous studies have only examined one-dose combinations. Much more work needs to be done to elucidate the mechanisms by which cannabinoids and opioids interact to produce analgesia. However, the implication that a combination of drugs may be more effective than either drug alone, and at the same time possibly reduce the occurrence of side effects, should provoke further study on analgesic drug interactions.
Acknowledgements

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References


Figure Legends

**Figure 1.** Dose-response curves for (A) ∆⁹-THC; (B) morphine and (C) codeine in the tail flick test for antinociception. Animals were tested 30 min following drug administration. Each point ± S.E. represents the mean response from a group of 6 mice. ED₅₀ values presented in Table 1 were determined by linear regression.

**Figure 2.** Isobologram of ∆⁹-THC/morphine drug combinations. The points designated z₁ and z₂ represent the ED₅₀ values for each drug alone, and the line connecting these points contains all dose pairs that are simply additive. Points A and B represent the theoretically additive value for the combinations z₁:z₂ and 0.1z₁:0.9z₂, respectively. Point C represents the experimentally determined value for the combination z₁:z₂ and point D represents the experimentally determined value for the combination 0.1z₁:0.9z₂. Since both points C and D fall to left and below the line of theoretical additivity, they indicate synergy between ∆⁹-THC and morphine.

**Figure 3.** Isobologram of ∆⁹-THC/codeine drug combinations. The points designated z₁ and z₃ represent the ED₅₀ values for each drug alone, and the line connecting these points contains all dose pairs that are simply additive. Points A and B represent the theoretically additive value for the combinations z₁:z₃ and 0.2z₁:0.8z₃, respectively. Point C represents the experimentally determined value for the combination z₁:z₃ and point D represents the experimentally determined value for the combination 0.2z₁:0.8z₃. Since both points C and D fall to left and below the line of theoretical additivity, they indicate synergy between ∆⁹-THC and codeine.
Table 1. ED$_{50}$ values ± S.E. for Δ$^9$-THC p.o., morphine p.o. and codeine p.o. in the tail-flick test for antinociception.

<table>
<thead>
<tr>
<th>THC (mg/kg)</th>
<th>Morphine (mg/kg)</th>
<th>Codeine (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$z_1 = 89.4 \pm 19.9$</td>
<td>$z_2 = 24.5 \pm 4.8$</td>
<td>$z_3 = 78.2 \pm 14.4$</td>
</tr>
<tr>
<td>$\log (z_1) = 1.95 \pm 0.097$</td>
<td>$\log (z_2) = 1.39 \pm 0.085$</td>
<td>$\log (z_3) = 1.89 \pm 0.080$</td>
</tr>
</tbody>
</table>
Table 2. Amounts of $\Delta^9$-THC and morphine in fixed ratio mixtures and corresponding additive amounts.

<table>
<thead>
<tr>
<th>Proportion (THC/Mor)</th>
<th>Experimental $Z_{\text{mix}}$</th>
<th>log ($Z_{\text{mix}}$)</th>
<th>Additive $Z_{\text{add}}$</th>
<th>log ($Z_{\text{add}}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$z_1 : z_2$</td>
<td>13.6 ± 1.94</td>
<td>1.13 ± 0.062*</td>
<td>57.0 ± 10.2</td>
<td>1.76 ± 0.078</td>
</tr>
<tr>
<td>0.1$z_1 : 0.9z_2$</td>
<td>12.7 ± 1.33</td>
<td>1.11 ± 0.045*</td>
<td>31.0 ± 4.76</td>
<td>1.49 ± 0.067</td>
</tr>
</tbody>
</table>

Amounts are total (in mg/kg) in the mixture to produce 50% effect, and are given along with logarithmic values and standard errors. * Significant, $p<0.05$ from corresponding additive values.
Table 3. Amounts of Δ⁹-THC and codeine in fixed ratio mixtures and corresponding additive amounts.

<table>
<thead>
<tr>
<th>Proportion (THC/Cod)</th>
<th>Experimental Mixtures</th>
<th>Log (Zmix)</th>
<th>Additive (theoretical)</th>
<th>Log (Zadd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>z₁:z₃</td>
<td>20.1 ± 3.00</td>
<td>1.30 ± 0.065⁺</td>
<td>83.8 ± 12.3</td>
<td>1.92 ± 0.064</td>
</tr>
<tr>
<td>0.2z₁:0.8z₃</td>
<td>45.6 ± 3.99</td>
<td>1.66 ± 0.038⁺</td>
<td>80.5 ± 12.2</td>
<td>1.91 ± 0.066</td>
</tr>
</tbody>
</table>

Amounts are total (in mg/kg) in the mixture to produce 50% effect, and are given along with logarithmic values and standard errors. ⁺ Significant, p<0.05 from corresponding additive values.