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Synergistic effects between codeine and diclofenac after local, spinal and systemic administration

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Abstract

This study was designed to evaluate the extent of the antinociceptive interaction between codeine and diclofenac at the local, spinal and systemic level. The effects of individual and fixed-ratio combinations of locally, spinally or orally given codeine and diclofenac were assayed using the formalin test in rats. Isobolographic analysis was employed to characterize the synergism produced by the combinations. Codeine, diclofenac and fixed-ratio codeine–diclofenac combinations produced a dose-dependent antinociceptive effect when administered locally, spinally or systemically. ED_{30} values were estimated for the individual drugs and isobolograms were constructed. Theoretical ED_{30} values for the combination estimated from the isobolograms were 422.2 ± 50.5 μg/paw, 138.5 ± 9.2 μg/rat, and 9.3 ± 1.1 mg/kg for the local, spinal and oral routes, respectively. These values were significantly higher than the actually observed ED_{30} values which were 211.1 ± 13.6 μg/paw, 45.9 ± 3.9 μg/rat, and 2.5 ± 0.2 mg/kg, indicating a synergistic interaction. Systemic administration resulted in the highest increase in potency, being about fourfold, while spinal and local administration increased potency in two- and threefold, respectively. The fact that the highest synergism was observed after systemic administration suggests that the interaction is occurring at several anatomical sites. The results support the clinical use of this combination in pain management.

Keywords: Synergistic; Codeine; Diclofenac; Antinociception

1. Introduction

Combinations of nonsteroidal anti-inflammatory drugs (NSAIDs) with opioids are currently used in clinical practice to reduce opioid requirements (Reasbeck et al., 1982; Burns et al., 1991; Kehlet and Dahl, 1993). The purpose is to improve analgesia without enhancing the side effects of each drug. Accordingly, clinical studies have described a 20–50% reduction in the opioid requirement when NSAIDs are added (Kehlet and Dahl, 1993). Experimental studies have reported a synergism between intravenous morphine and diclofenac, but only an additive interaction between morphine with propacetamol in an inflammatory pain model in rats (Fletcher et al., 1997). On the other hand, the morphine–ketorolac combination has shown a significant synergism in the formalin, visceral nociception and neuropathic pain tests (Malmberg and Yaksh, 1993; Maves et al., 1994; Lashbrook et al., 1999). Moreover, acetylsalicylic acid significantly increased the antinociceptive effect of morphine in the hot-plate and formalin tests (Sandrini et al., 1998), whereas that local administration of dipyrone increased the peripheral antinociceptive effect of morphine in the formalin test (Aguirre-Bañuelos and Granados-Soto, 1999). Notwithstanding these observations, the information regarding the potential benefit of NSAID-opioid combinations yielding a rational basis for their use in clinical practice is still scarce.

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Codeine has been widely used in the management of clinical postoperative pain, alone and combined with acetaminophen (Forbes et al., 1990; Dhaliwal et al., 1995; De Craen et al., 1996; Poulsen et al., 1998; Innes et al., 1998). However, recent evidence suggest that combinations of codeine and acetaminophen are not well tolerated and do not offer a superior alternative for pain control (De Craen et al., 1996; Eckhardt et al., 1998; Chang et al., 2001). Therefore, other combinations have been explored. A clinical study showed that the codeine–diclofenac combination produced a better analgesic response than the diclofenac alone (Strobel, 1992). However, another study did not find an increased analgesic effect with this combination of analgesic agents in cancer pain (Minotti et al., 1998). To gain more insight on the antinociceptive efficacy of codeine–diclofenac combinations, the current study was designed to assess the peripheral, spinal and systemic antinociceptive effect of codeine and diclofenac and their possible synergistic interaction by isobolographic analyses in the rat formalin test.

2. Methods

2.1. Animals

All experiments were conducted in accordance with the "Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals" (Zimmermann, 1983). In addition, the study was approved by the Institutional Animal Care and Use Committee (Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City, Mexico). Male Wistar rats aged 6–7 weeks (weight range, 160–200 g) from our own breeding facilities were used in this study. Animals had access to food and drinking water ad libitum before the experiments, except those included in the systemic drug administration protocol that were allowed only water 12 h before the experiments.

2.2. Spinal surgery

For spinal administration, rats were anesthetized with a ketamine/xylazine mixture (50/20 mg/kg ip), then were placed in a stereotaxic head holder, and the atlanto-occipital membrane was exposed (Malmberg and Yaksh, 1992). The membrane was pierced, and a PE-10 catheter was passed intrathecally to the level of the thoracolumbar junction and the wound was sutured. Rats were allowed to recover from surgery for at least 5 days before use. Animals showing any signs of motor impairment were euthanized in a CO_2 chamber.

2.3. Measurement of antinociceptive activity

Antinociception was assessed using the formalin test (Malmberg and Yaksh, 1992). Rats were placed in open Plexiglas observation chambers for 30 min to allow them to accommodate to their surroundings, then they were removed for formalin administration. Fifty microliters of diluted formalin (5%) was injected subcutaneously into the dorsal surface of the right hind paw with a 30-gauge needle. Animals were then returned to the chambers, and nociceptive behavior was observed immediately after formalin injection. Mirrors were placed to enable unhindered observation. Nociceptive behavior was quantified as the number of flinches of the injected paw during 1-min periods every 5 to 60 min after injection (Malmberg and Yaksh, 1992; Wheeler-Aceto and Cowan, 1991). Flinching was readily discriminated and was characterized as rapid and brief withdrawal or flexing of the injected paw. Formalin-induced flinching behavior is biphasic. The initial acute phase (0–10 min) is followed by a relatively short quiescent period, which is then followed by a prolonged tonic response (15–60 min). Both phases of the formalin test were registered. Notwithstanding, since one of the tested drugs, diclofenac, was only active on the second phase, further data analysis are based on the results of this phase. At the end of the experiment the rats were sacrificed in a CO_2 chamber.

2.4. Drugs

Codeine phosphate and diclofenac sodium were kindly supplied by Novartis Farmacéutica (Mexico City, Mexico). Both analgesic agents were dissolved in saline and administered either spinally (in 10 μl) or subcutaneously (in 50 μl). For the oral administration, drugs were suspended in carboxymethylcellulose 0.5% and given at a volume ratio of 4 ml/kg.

2.5. Study design

For the local study, rats received a subcutaneous injection (50 μl) in the dorsal surface of the right hind paw of vehicle or increasing doses of either codeine (100–1500 μg/paw), diclofenac (100–1000 μg/paw) or the codeine–diclofenac combination (as indicated in Table 1) 20 min before formalin injection in the same paw (ipsilateral). To assess if the antinociceptive effect of drugs was due to a local action, formalin was administered in one paw and the greatest dose of the tested drugs in the contralateral paw. For the spinal study, rats were intrathecally injected with increasing doses of codeine (25–400 μg), diclofenac (25–200 μg) or the combination codeine–diclofenac (as indicated in Table 1) 20 min before formalin injection. In the systemic study, animals received increasing doses of codeine (1–100 mg/kg), diclofenac (1–32 mg/kg) or the codeine–diclofenac combinations (as indicated in Table 1) orally 20 min before formalin injection. For all routes of administration, doses were selected on the basis of pilot as well as previous studies in our model (Ortiz et al., 2002). The observer was unaware of the treatment in each animal. Rats in all groups were tested for possible side effects such

Table 1
Doses used in the study of the interaction between codeine and diclofenac after local, spinal and systemic administration to rats in the formalin test

Local dose (μg/paw)			Spinal dose (μg/rat)			Systemic dose (mg/kg)		
Codeine in the combination	Diclofenac in the combination	Total dose in the combination	Codeine in the combination	Diclofenac in the combination	Total dose in the combination	Codeine in the combination	Diclofenac in the combination	Total dose in the combination
24.1	28.6	52.7	8.5	8.7	17.3	0.63	0.53	1.1
48.2	57.2	105.5	17.1	17.5	34.6	1.2	1.0	2.3
96.5	114.5	211.1	34.2	35	69.2	2.5	2.1	4.6
193	229	422.2	68.5	70	138.5	5.0	4.3	9.3
–	–	–	137	140	277	10.0	8.6	18.6

as reduction of righting, stepping, corneal and pinna reflexes before and after drug treatment.

2.6. Data analysis

All results are presented as mean ± S.E.M. for at least six animals per group. Time-courses of antinociceptive response of individual drugs and combinations were constructed by plotting the mean number of flinches as a function of time. The total sum of flinches corresponding to the second phase of the assay was determined from 15 to 60 min, with regard to formalin administration. Dose–response data are presented as the percent of antinociception of the total sum of flinches on the second phase of the formalin test. Percent of antinociception was calculated according to the following equation (Argüelles et al., 2002):

$$\text{Percent of Antinociception}$$

$$[(\text{vehicle} - \text{post compound})/\text{vehicle}] \times 100.$$

The dose–response curves were constructed and the experimental points fitted using least-squares linear regression. ED_{30} ± standard error (S.E.M.) was calculated according to the method described by Tallarida (2000).

It has been previously demonstrated that, for evaluation of the interaction between analgesic drugs, isobolographic analysis is a convenient tool (Argüelles et al., 2002; Tallarida, 2000). Therefore, in the present study, we used such technique to determine the nature of drug interaction between codeine and diclofenac. Isobolographic analysis assumes that the combination of drugs is made from equipotent doses of the individual drugs. Thus, from the dose–response curves of each individual agent, the dose resulting in 50% of the effect (ED_{50}) can be determined. However, considering a maximal effect of 100% as the total suppression of formalin-induced flinches, it appeared that diclofenac was unable to achieve a 50% response, and thus the calculation of ED_{50} was not feasible. Therefore, we estimated the ED_{30} instead of the ED_{50}. Subsequently, a dose–response curve was obtained by concurrent delivery of the two drugs in a constant dose ratio (fixed-ratio) based on the ED_{30} values of each individual agent. To construct these curves, five groups of animals were formed and each group received one of the following doses of the combination: codeine ED_{30} + diclofenac ED_{30}; (codeine ED_{30} + diclofenac ED_{30})/2; (codeine ED_{30} + diclofenac ED_{30})/4; (codeine ED_{30} + diclofenac ED_{30})/8; and (codeine

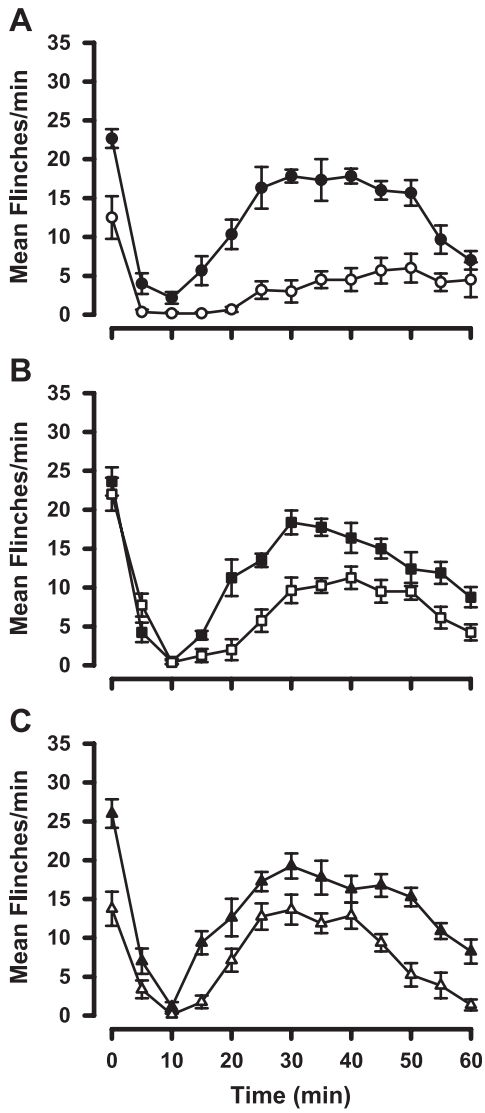

Fig. 1. Time-course of the antinociceptive effect of local (peripheral) administration of (A) codeine (1500 μg/paw; ○) and vehicle (●); (B) diclofenac (1000 μg/paw; □) and vehicle (■); and (C) codeine–diclofenac (230:193 μg/paw; △) and vehicle (▲). Data represent the mean ± S.E.M. of at least six rats.

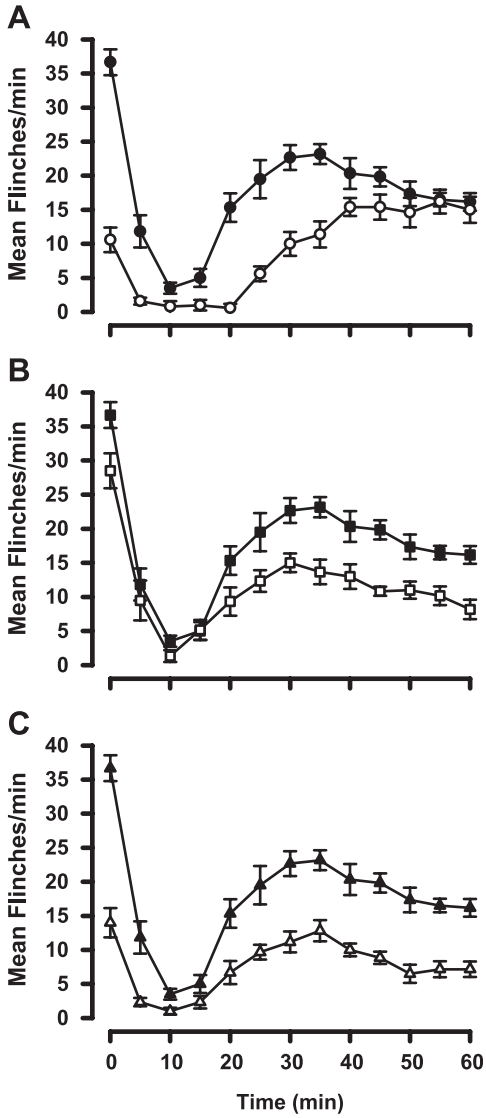

Fig. 2. Time-course of the antinociceptive effect of spinal administration of (A) Codeine (400 μg/rat; ○) and vehicle (●); (B) diclofenac (200 μg/rat; □) and vehicle (■); and (C) codeine–diclofenac (140:137 μg/rat; △) and vehicle (▲). Data represent the mean ± S.E.M. of at least six rats.

ED_{30} + diclofenac ED_{30})/16. Detailed information on the composition of different combinations used in this study is shown in Table 1. From the resulting dose–response curve of the combination, the experimental ED_{30} value was calculated.

To determine if the interaction between two drugs given in combination was synergistic, additive or antagonistic, the theoretical additive ED_{30} was estimated from the dose–response curves of each drug administered individually, i.e., considering that the observed effect with the combination results of the sum of the individual effects of each component. This theoretical ED_{30} value is then compared with the experimental ED_{30} to determine if there is a statistically significant difference (Tallarida, 2002; Tallarida et al., 1999).

The theoretical and experimental ED_{30} values of the studied of combinations were also contrasted by calculating the interaction index (γ) as follows:

$$\gamma = ED_{30} \text{ of combination (experimental)} \; / ED_{30} \text{ of combination (theoretical)}$$

The interaction index indicates what portion of the ED_{30} of the individual drugs accounts for the corresponding ED_{30} in the combination, i.e., values near to 1 correspond to an additive interaction, values higher than 1 imply an antagonistic interaction, and values lower than 1 indicate a synergistic interaction.

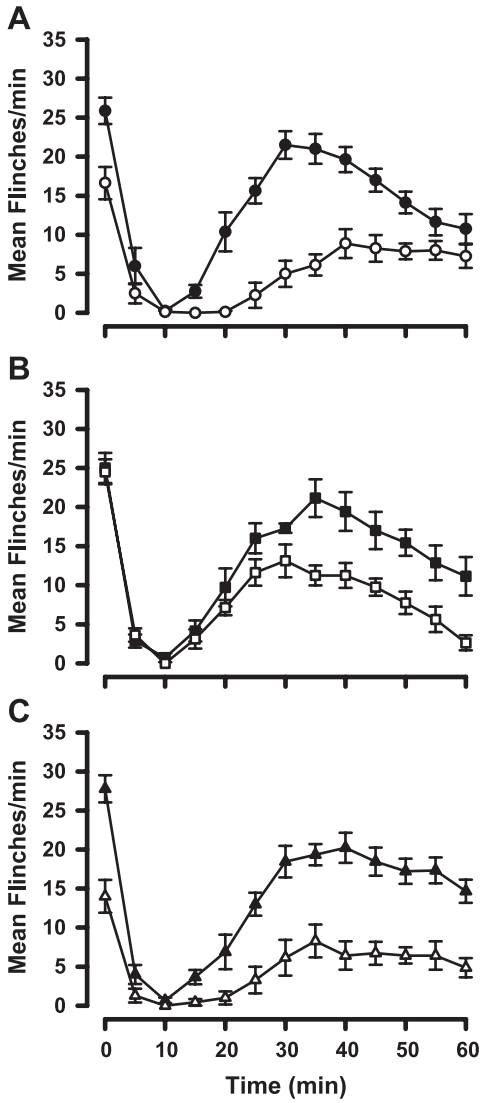

Fig. 3. Time-course of the antinociceptive effect of systemic (oral) administration of (A) codeine (100 mg/kg; ○) and vehicle (●); (B) diclofenac (32 mg/kg; □) and vehicle (■); and (C) codeine–diclofenac (8.6:10 mg/kg; △) and vehicle (▲). Data represent the mean ± S.E.M. of at least six rats.

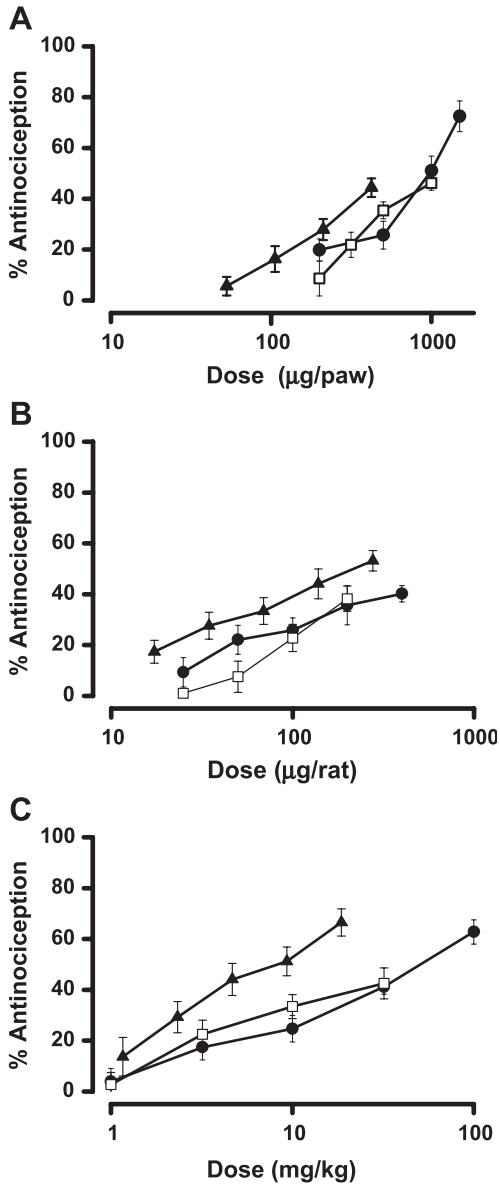

Fig. 4. Comparative dose–effect curves for the local (A), spinal (B) and systemic (C) administration of codeine (●), diclofenac (□) and diclofenac–codeine (▲) during the second phase of the formalin test. Doses of codeine were 200, 500, 1000 and 1500 µg/paw, 25, 50, 100, 200 and 400 µg/rat, and 1, 3.2, 10, 32 and 100 mg/kg for peripheral, spinal and, systemic administration, respectively. Doses of diclofenac were 200, 375, 500 and 1000 µg/paw, 25, 50, 100, 200 µg/rat, and 1, 3.2, 10 and 32 mg/kg for peripheral, spinal and systemic administration, respectively. Total doses of the codeine–diclofenac combination were 52.7, 105.5, 211.1 and 422.2 µg/paw, 17.3, 33.6, 69.2, 138.5 and 277.0 µg/rat, and 1.1, 2.3, 4.6, 9.3 and 18.6 mg/kg for peripheral, spinal, and systemic administration, respectively. Each point corresponds to the mean ± S.E.M. of at least six rats.

2.7. Statistical analysis

Dose–response data were analyzed by one-way analysis of variance (ANOVA) with Dunnet's test for post hoc comparison. Statistical significance between the theoretical additive ED_{30} and the experimentally derived ED_{30} values was evaluated using Student's *t* test (Tallarida, 2000). An experimental ED_{30} value significantly lower than the theoretical additive ED_{30} was considered to indicate a synergistic interaction between codeine and diclofenac. Statistical significance was considered to be achieved when $P < .05$.

3. Results

3.1. Peripheral, spinal and systemic antinociceptive effect of codeine, diclofenac and combinations

Subcutaneous injection of formalin into the hind paw produced a typical pattern of flinching behavior. The first phase started immediately after administration of formalin and then diminished gradually in approximately 10 min. The second phase started at 15 min and lasted until 1 h (Aguirre-Bañuelos and Granados-Soto, 1999). In the second phase of the formalin test, both codeine and diclofenac induced a dose-dependent antinociceptive effect by the three studied routes of administration (Figs. 1–3). No differences in the measured reflexes were observed before and after treatment in either group, control or treated, in any of the studied routes of administration. Figs. 1–3 show the time-courses of flinching behavior in control and treated rats with highest doses assayed of codeine, diclofenac and the codeine–diclofenac combination given by different routes. Local ipsilateral, but not contralateral, spinal or oral administration of individual or combined drugs produced a significant ($P < .05$) reduction in the flinching behavior otherwise observed after formalin injection, although only codeine and the combination were active on the first phase of the assay. Thus, only data from the second phase of the assay were submitted to further analysis.

3.2. Interaction of codeine and diclofenac after peripheral, spinal and systemic administration

Dose–response curves for codeine and diclofenac were constructed (Fig. 4) and the dose producing 30% of the

Table 2
Effect of peripheral, spinal and oral administration of codeine and diclofenac alone or in combination in the formalin test

	Peripheral (local) administration ED_{30}, µg/paw	Spinal administration ED_{30}, µg/rat	Systemic administration ED_{30}, mg/kg
Codeine	385.6 ± 96.1	136.8 ± 14.9	10.0 ± 1.7
Diclofenac	458.5 ± 31.3	140.0 ± 10.8	8.6 ± 1.5
Theoretical combination	422.2 ± 50.5	138.5 ± 9.2	9.3 ± 1.1
Experimental combination	211.1 ± 13.6 *	45.9 ± 3.9 *	2.5 ± 0.2 *
Interaction index	0.50 ± 0.06	0.33 ± 0.03	0.27 ± 0.04

ED_{30}: Effective dose resulting in a 30% reduction on control response. Data are the mean ± S.E. of the estimate.

* Significantly different from the theoretical combination data ($P < .05$), by the Student's *t* test.

maximal antinociceptive effect (ED_{30}) was estimated. The doses of the codeine–diclofenac association were prepared as fixed-dose ratio dilutions with respect to the ED_{30} values of each individual agent (Table 1), and were used to construct dose–response curves for all the studied routes of administration (Fig. 4). This strategy allowed the estimation of the experimental ED_{30} values for the combinations (Table 2). A leftward shift of the dose–response curve of the codeine–diclofenac combination, indicating increased antinociceptive potency with regard to the individual drugs, was observed with all routes of administration, although it was more pronounced when drugs were given orally (Fig. 4C).

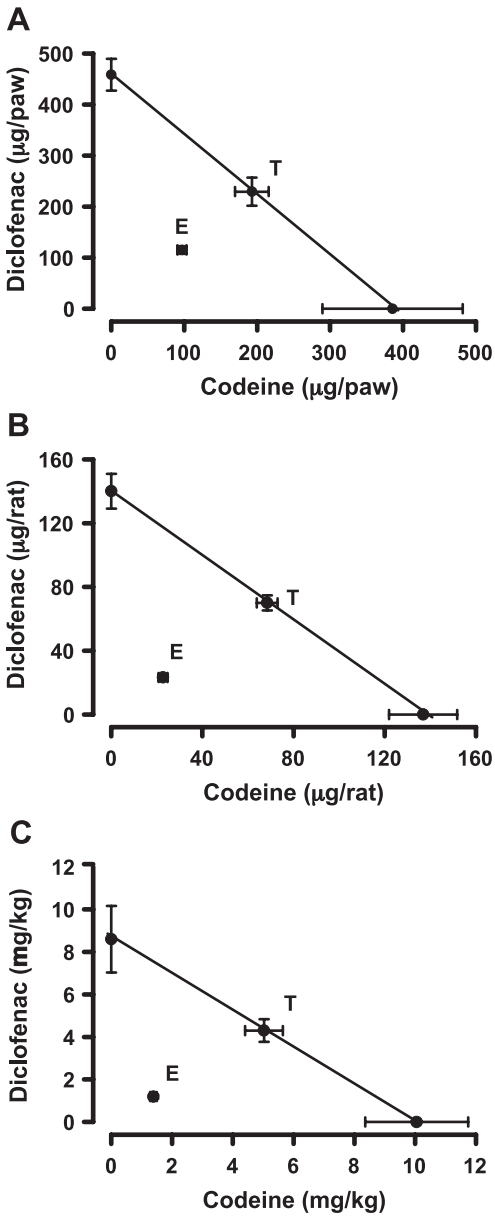

Fig. 5. Isobolograms showing the local (A), spinal (B) and systemic (C) interaction between diclofenac and codeine in the formalin test. Horizontal and vertical bars indicate S.E.M. The oblique line between the *x* and *y* axes is the theoretical additive line. The point in the middle of this line, indicated by "T", is the theoretical additive point calculated from the individual drug ED_{30} values. The point indicated by "E" is the actually observed ED_{30} value with the combination. In all cases, the experimental ED_{30} point is situated below the additive line, being statistically significantly different for the theoretical ED_{30} value assuming a purely additive interaction, indicating a significant synergism (P=.05).

When the ED_{30} values were submitted to isobolographic analysis, it appeared that the experimental values were lower than those expected from a purely additive interaction. This can be graphically appreciated in Fig. 5. The theoretically additive dose line depicts all points of codeine–diclofenac dose combinations yielding an effect of 30% according to a purely additive interaction. Thus, the point corresponding to the codeine and diclofenac amounts actually given in the combination located on this line corresponds to the theoretical ED_{30} value. For the three studied routes of administration, the experimental ED_{30} values of the codeine–diclofenac combination clearly were situated below theoretically additive dose line, indicating a synergistic interaction between codeine and diclofenac after peripheral, spinal and oral administration (Fig. 5A–C). Comparison of experimental and theoretical ED_{30} values by the Student's *t* test yielded statistically significant differences ($P<.05$) for all routes (Table 2). Further analysis according to the interaction index values (Table 2), showed a twofold increase in potency was achieved by the local route ($\gamma=0.5$), a threefold increase was reached by the spinal route ($\gamma=0.33$) and a near fourfold increase was found by the systemic route ($\gamma=0.27$).

4. Discussion

The current study demonstrates that peripherally, spinally or orally administered codeine produced dose-dependent antinociception in the formalin test. The antinociceptive effect of codeine has been demonstrated in acute thermal pain models and in prostaglandin-induced hyperalgesia (Yaksh and Rudy, 1976; Kamata et al., 1980; Molina et al., 1983). Therefore, our results confirm previous observations indicating that codeine produce antinociception after peripheral, spinal, or systemic administration (Yaksh and Rudy, 1976; Kamata et al., 1980; Molina et al., 1983; Srinivasan et al., 1996) in several models of pain. It has been assumed that codeine is an analgesic prodrug of morphine (Sanfilippo, 1948; Pert and Snyder, 1973). Therefore, it is likely that codeine-induced antinociception is due to activation of opioid receptors.

On the other hand, diclofenac is known to cause antinociception after peripheral and systemic administration to animals in several models of pain (Ortiz et al., 2002; Menassé et al., 1978; Tonussi and Ferreira, 1993; Torres-López et al., 1997, 2002; Asomoza-Espinosa et al., 2001) as well as in clinical pain in humans (Todd and Sorkin, 1988). In our study, diclofenac administration at the peripheral and systemic level produced a dose-related antinociception in the formalin test, but only during the second phase of the

assay. These results demonstrate the antinociceptive efficacy of diclofenac, and are consistent with a significant participation of a peripheral component in its mechanism of action on formalin-induced nociception. In the present work, we also demonstrated that diclofenac is able to produce antinociception by acting on the spinal cord. Our data agree with previous observations about the spinal antinociceptive efficacy of diclofenac in rats (Miranda et al., 2001a,b; Pinardi et al., 2002). Since diclofenac is an inhibitor of prostaglandin synthesis (Kato et al., 2001), our data suggest that diclofenac could be reducing prostaglandin-induced sensitization in the primary afferent neurons and at the spinal cord, although the participation of additional mechanisms cannot be ruled out.

The present study focused on the nature of the interaction between codeine and diclofenac at several levels. Conceptually, an additive effect refers to the interaction between two drugs such that, when coadministered, the resultant effect approaches the maximum effect or the sum the effects of the two drugs administered individually. Synergy describes the interaction between two drugs such that, when given concurrently, the resultant efficacy or potency supports a greater-than-additive or multiplicative interaction compared to each drug administered individually (see Tallarida, 2000, for a more detailed review). In this study, by using a fixed-ratio strategy, isobolographic analysis demonstrated a significant synergistic interaction between codeine and diclofenac at peripheral, spinal and systemic levels. These results confirm previous experiments showing that coadministration of opioids and NSAIDs to rats produce an increased peripheral (Aguirre-Bañuelos and Granados-Soto, 1999), spinal (Malmberg and Yaksh, 1993) and systemic (Fletcher et al., 1997) antinociceptive effect compared with individual drugs. However, to our knowledge, this is the first report about the synergistic interaction between codeine and diclofenac in the rat at different levels of pain transmission.

The mechanism underlying the synergism between codeine and diclofenac is not clear. A pharmacokinetic interaction seems to be unlikely, since there is data that administration of diclofenac does not modify the plasma levels of codeine or its metabolites, including morphine (Ammon et al., 2002). A pharmacodynamic interaction appears more plausible. The mechanism of the observed synergism could be due to the different sites of action of diclofenac and codeine as well as to the multiple mechanisms of antinociceptive action of both drugs. Diclofenac exhibits mechanisms additional to nonselective cyclooxygenase inhibition, which have been proposed to play a significant role in the antinociceptive effect of this NSAID. Thus, activation of the serotonergic inhibitory descendent system, reduction of the pronociceptive actions of glutamate at the spinal cord, activation of α_2-adrenoceptors at spinal and supraspinal levels, and activation of K^+ channels also appear to be involved in the antinociceptive effect of diclofenac in the formalin test (Björkman, 1995; Ortiz et al., 2002; Pinardi et al., 2002). Furthermore, diclofenac increases the hypothalamic levels of β-endorphins (Sacerdote et al., 1985), possibly increasing the activity of the opioid system when both agents are administered concomitantly. On the other hand, the antinociception produced by codeine likely results from activation of opioid receptors, by either morphine or unchanged codeine (Sanfilippo, 1948; Pert and Snyder, 1973; Quiding et al., 1993). Notwithstanding, a direct antinociceptive action of codeine by other mechanism cannot be ruled out (Quiding et al., 1993). The first view has been supported by experiments showing that codeine binds to opioid receptors, but with a much lower affinity compared to morphine (Pert and Snyder, 1973). There is evidence showing that μ-opioid receptor agonists have antinociceptive activity in the formalin test after peripheral, spinal and systemic administration (Yaksh and Rudy, 1976; Antonijevic et al., 1995; Granados-Soto et al., 1997; Shannon and Lutz, 2002). It is presently known that the effect of opioids involve several mechanisms. It has been described that μ-opioid receptor agonists act to inhibit activation of adenylyl cyclase (Ingram and Williams, 1996) and tetrodotoxin-resistant Na^+ channels on peripheral afferent neurons produced by inflammatory mediators such as prostaglandin E_2 and serotonin (Gold et al., 1996). Moreover, there is also evidence pointing that opioids inhibit release of substance P and calcitonin gene-related peptide from primary afferent neurons (Yaksh, 1988), and open ATP-sensitive K^+ channels via Gi proteins resulting in hyperpolarization, reduction in firing of the primary afferent neuron and antinociception (Ocaña et al., 1990; Ortiz et al., 2002; Rodrigues and Duarte, 2000; Yoshimura and North, 1983). All or some of these mechanisms could be involved in the antinociceptive effect of codeine at the peripheral, spinal o supraspinal level. Which of these mechanisms are actually involved in the synergistic interaction between diclofenac and codeine, however, remains to be elucidated.

Previous studies have shown that the concurrent use of opioids and NSAIDs produces increased antinociception or a reduction in the requirements of opioid agents (Kehlet and Dahl, 1993; Rockeman et al., 1996; Tallarida et al., 1999; Silvanto et al., 2002). Nevertheless, the clinical use of a combination of codeine and diclofenac is still controversial. A clinical study suggested that a combination 50 mg of codeine and 50 mg of diclofenac showed a better analgesic response than the diclofenac alone (Strobel, 1992), while the combination of 40 mg of codeine and 50 mg of diclofenac was unable to improve the analgesic response of diclofenac alone (Minotti et al., 1998). Our results in the rat showed that it is possible to observe a synergistic interaction between codeine and diclofenac at different anatomical sites. This is supported by previous electrophysiological observations showing that opioids produce antinociception by suppressing the inhibitory influence of GABA on neurons constituting a descending antinociceptive pathway, and that such effect is potentiated by cyclooxygenase-1 inhibitors (Vaughan et al., 1997; Vaughan, 1998). It has been

proposed that this mechanism accounts for the antinociceptive activity of cyclooxygenase inhibitors in the periaqueductal gray, as well as for NSAID synergism with opioids (Vaughan et al., 1997).

In conclusion, the present study has demonstrated that codeine and diclofenac produce antinociception in the formalin test after peripheral, spinal and systemic administration. Moreover, the existence of an important functional synergistic interaction between codeine and diclofenac at different levels of pain transmission is documented. Therefore, clinical studies assessing the therapeutic potential of this combination are encouraged.

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References

Aguirre-Bañuelos P, Granados-Soto V. Evidence for a peripheral mechanism of action for the potentiation of the antinociceptive effect of morphine by dipyrone. J Pharmacol Toxicol Meth 1999;42:79–85.

Ammon S, Marx C, Behrens C, Hofmann U, Murdter T, Griese EU, et al. Diclofenac does not interact with codeine metabolism in vivo: a study in healthy volunteers. BMC Clin Pharmacol 2002;2:2–12.

Antonijevic I, Mousa SA, Schafer M, Stein C. Perineural defect and peripheral opioid analgesia in inflammation. J Neurosci 1995;15:165–72.

Argüelles CF, Torres-López JE, Granados-Soto V. Peripheral antinociceptive action of morphine and the synergistic interaction with lamotrigine. Anesthesiology 2002;96:921–5.

Asomoza-Espinosa R, Alonso-López R, Mixcoatl-Zecuatl T, Aguirre-Bañuelos P, Torres-López JE, Granados-Soto V. Sildenafil increases diclofenac antinociception in the formalin test. Eur J Pharmacol 2001;418:195–200.

Björkman R. Central antinociceptive effects of non-steroidal anti-inflammatory drugs and paracetamol. Experimental studies in the rat. Acta Anaesthesiol Scand Suppl 1995;103:1–44.

Burns JW, Aitken HA, Bullingham RES, McArdle CS, Kenny GNC. Double-blind comparison of the morphine sparing effect of continuous and intermittent i.m. administration of ketorolac. Br J Anaesth 1991;67:235–8.

Chang DJ, Fricke JR, Bird SR, Bohidar NR, Dobbins TW, Geba GP. Rofecoxib versus codeine/acetaminophen in postoperative dental pain: a double-blind, randomized, placebo- and active comparator-controlled clinical trial. Clin Ther 2001;23:1446–55.

De Craen A, Di Giulio G, Lampe-Schoenmaeckers AJ, Kessels AG, Kleijnen J. Analgesic efficacy and safety of paracetamol–codeine combinations versus paracetamol alone: a systematic review. Br Med J 1996;313:321–5.

Dhaliwal HS, Sloan P, Arkinstall WW, Thirlwell MP, Harsanyi Z, Darke AC. Randomized evaluation of controlled-release codeine and placebo in chronic cancer pain. J Pain Symptom Manag 1995;10:612–23.

Eckhardt K, Li S, Ammon S, Schanzle G, Mikus G, Eichelbaum M. Same incidence of adverse drug events after codeine administration irrespective of the genetically determined differences in morphine formation. Pain 1998;76:27–33.

Fletcher D, Benoist JM, Gautron M, Guilbaud G. Isobolographic analysis of interactions between intravenous morphine, propacetamol, and diclofenac in carrageenin-injected rats. Anesthesiology 1997;87:317–26.

Forbes JA, Kehm CJ, Grodin CD, Beaver WT. Evaluation of ketorolac, ibuprofen, acetaminophen, and an acetaminophen–codeine combination in postoperative oral surgery pain. Pharmacotherapy 1990;10:94S–105S.

Gold MS, Reichling DB, Shuster MJ, Levine JD. Hyperalgesic agents increase a tetrodotoxin-resistant Na^+ current in nociceptors. Proc Natl Acad Sci U S A 1996;93:1108–12.

Granados-Soto V, Rufino MO, Gomes Lopes LD, Ferreira SH. Evidence for the involvement of the nitric oxide-cGMP pathway in the antinociception of morphine in the formalin test. Eur J Pharmacol 1997;340:177–80.

Ingram SL, Williams JT. Modulation of the hyperpolarization-activated current (Ih) by cyclic nucleotides in guinea-pig primary afferent neurons. J Physiol 1996;492:97–106.

Innes GD, Croskerry P, Worthington J, Beveridge R, Jones D. Ketorolac versus acetaminophen–codeine in the emergency department treatment of acute low back pain. J Emerg Med 1998;16:549–56.

Kamata K, Okuyama S, Kameyama T. Effects of analgesics and CNS-acting drugs on struggling following repetitive stimulation of the tail, and flexor reflex to a single stimulation of the sciatic nerve in rats. Jpn J Pharmacol 1980;30:357–66.

Kato M, Nishida S, Kitasato H, Sakata N, Kawai S. Cyclooxygenase-1 and cyclooxygenase-2 selectivity of non-steroidal anti-inflammatory drugs: investigation using human peripheral monocytes. J Pharm Pharmacol 2001;53:1679–85.

Kehlet H, Dahl JB. The value of "multimodal" or "balanced analgesia" in postoperative pain treatment. Anesth Analg 1993;77:1048–56.

Lashbrook JM, Ossipov MH, Hunter JC, Raffa RB, Tallarida RJ, Porreca F. Synergistic antiallodynic effects of spinal morphine with ketorolac and selective COX1- and COX2-inhibitors in nerve-injured rats. Pain 1999;82:65–72.

Malmberg AB, Yaksh TL. Antinociceptive actions of spinal nonsteroidal anti-inflammatory agents on the formalin test in the rat. J Pharmacol Exp Ther 1992;263:136–46.

Malmberg AB, Yaksh TL. Pharmacology of the spinal action of ketorolac, morphine, ST-91, U50488H, and L-PIA on the formalin test and an isobolographic analysis of the NSAID interaction. Anesthesiology 1993;79:270–81.

Maves TJ, Pechman PS, Meller ST, Gebhart GF. Ketorolac potentiates morphine antinociception during visceral nociception in the rat. Anesthesiology 1994;80:1094–101.

Menassé R, Hedwall PR, Kraetz J, Pericin C, Riesterer L, Sallmann A, et al. Pharmacological properties of diclofenac sodium and its metabolites. Scand J Rheumatol Suppl 1978;22:5–16.

Minotti V, De Angelis V, Righetti E, Celani MG, Rossetti R, Lupatelli M, et al. Double-blind evaluation of short-term analgesic efficacy of orally administered diclofenac, diclofenac plus codeine, and diclofenac plus imipramine in chronic cancer pain. Pain 1998;74:133–7.

Miranda HF, Sierralta F, Pinardi G. An isobolographic analysis of the adrenergic modulation of diclofenac antinociception. Anesth Analg 2001a;93:430–5.

Miranda HF, Lopez J, Sierralta F, Correa A, Pinardi G. NSAID antinociception measured in a chemical and a thermal assay in mice. Pain Res Manag 2001b;6:190–6.

Molina N, Vettore O, Lorenzetti BB, Ferreira SH. The peripheral analgesic effect of morphine, codeine, pentazocine and d-propoxyphene. Braz J Med Biol Res 1983;16:345–52.

Ocaña M, Del Pozo E, Barrios M, Baeyens JM. An ATP-dependent K^+ channel blocker antagonizes morphine analgesia. Eur J Pharmacol 1990;186:377–8.

Ortiz MI, Torres-López JE, Castañeda-Hernández G, Rosas R, Vidal-Cantú GC, Granados-Soto V. Pharmacological evidence for the activation of K^+ channels by diclofenac. Eur J Pharmacol 2002;438:85–91.

Pert CB, Snyder SH. Properties of opiate-receptor binding in rat brain. Proc Natl Acad Sci U S A 1973;70:2243–7.

Pinardi G, Sierralta F, Miranda HF. Adrenergic mechanisms in antinociceptive effects of the non-steroidal anti-inflammatory drugs in acute thermal nociception in mice. Inflamm Res 2002;51:219–22.

Poulsen L, Riishede L, Brosen K, Clemensen S, Sindrup SH. Codeine in post-operative pain. Study of the influence of sparteine phenotype and serum concentrations of morphine and morphine-6-glucuronide. Eur J Clin Pharmacol 1998;54:451–4.

Quiding H, Lundqvist G, Boreus LO, Bondesson U, Ohrvik J. Analgesic effect and plasma concentrations of codeine and morphine after two dose levels of codeine following oral surgery. Eur J Clin Pharmacol 1993; 44:319–23.

Reasbeck PG, Rice ML, Raesbeck JC. Double blind controlled trial of indomethacin as an adjuvant to narcotic analgesia after major abdominal surgery. Lancet 1982;2:115–8.

Rockeman MG, Seeling W, Bischof C, Borstinghaus D, Steffen P, Georgieff M. Prophylactic use of epidural mepivacaine/morphine, systemic diclofenac and metamizole on postoperative morphine consumption after major abdominal surgery. Anesthesiology 1996;84:1027.

Rodrigues AR, Duarte ID. The peripheral antinociceptive effect induced by morphine is associated with ATP-sensitive K^+ channels. Br J Pharmacol 2000;129:110–4.

Sacerdote P, Monza G, Mantegazza P, Panerai AE. Diclofenac and pirprofen modify pituitary and hypothalamic beta-endorphin concentrations. Pharmacol Res Commun 1985;17:679–84.

Sandrini M, Ottani A, Vitale G, Pini LA. Acetylsalicylic acid potentiates the antinociceptive effect of morphine in the rat: involvement of the central serotonergic system. Eur J Pharmacol 1998;355:133–40.

Sanfilippo G. Contributo sperimentale all'ipotesi della smetilazione della codeine nell' organismo. I. Influence della dose sull'assuefazione alla codeine. II Assuetazione alla codeína ottenuta con somministrazione prolungata di morfina. Boll Ital Biol Sper 1948;24:723–6.

Shannon HE, Lutz EA. Comparison of the peripheral and central effects of the opioid agonists loperamide and morphine in the formalin test in rats. Neuropharmacology 2002;42:253–61.

Silvanto M, Lappi M, Rosenberg PH. Comparison of the opioid-sparing efficacy of diclofenac and ketoprofen for 3 days after knee arthroplasty. Acta Anaesthesiol Scand 2002;46:322–8.

Srinivasan V, Wielbo D, Simpkins J, Karlix J, Sloan K, Tebbett I. Analgesic and immunomodulatory effects of codeine and codeine 6-glucuronide. Pharm Res 1996;13:296–300.

Strobel E. Drug therapy in severe tumor pain. Comparative study of a new combination preparation versus diclofenac–Na. Fortschr Med 1992; 110:411–4.

Tallarida RJ. Drug synergism and dose–effect data analysis. 1st. ed. New York: Chapman & Hall/CRC; 2000. p 1–72.

Tallarida R.J. The interaction index: a measure of drug synergism. Pain 2002;98:163–8.

Tallarida RJ, Stone DJ, McCarty JD, Raffa RB. Response surface analysis of synergism between morphine and clonidine. J Pharmacol Exp Ther 1999;289:8–13.

Todd PA, Sorkin EM. Diclofenac sodium. A reappraisal of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. Drugs 1988;35:244–85.

Tonussi CR, Ferreira SH. Mechanism of diclofenac analgesia: direct blockade of inflammatory sensitization. Eur J Pharmacol 1993;251:173–9.

Torres-López JE, López-Muñoz FJ, Castañeda-Hernández G, Flores-Murrieta FJ, Granados-Soto V. Pharmacokinetic–pharmacodynamic modeling of the antinociceptive effect of diclofenac in rat. J Pharmacol Exp Ther 1997;282:685–90.

Torres-López JE, Ortiz MI, Castaneda-Hernandez G, Alonso-Lopez R, Asomoza-Espinosa R, Granados-Soto V. Comparison of the antinociceptive effect of celecoxib, diclofenac and resveratrol in the formalin test. Life Sci 2002;70:1669–76.

Vaughan CW. Enhancement of opioid inhibition of GABAergic synaptic transmission by cyclo-oxygenase inhibitors in rat periaqueductal gray neurons. Br J Pharmacol 1998;123:1478–81.

Vaughan CW, Ingram SL, Connor MA, Christie MJ. How opioids inhibit GABA-mediated neurotransmission. Nature 1997;390:611–4.

Wheeler-Aceto H, Cowan A. Standardization of the rat paw formalin test for the evaluation of analgesics. Psychopharmacology 1991;104:35–44.

Yaksh TL. Substance P release from knee joint afferent terminals: modulation by opioids. Brain Res 1988;458:319–24.

Yaksh TL, Rudy TA. Analgesia mediated by a direct spinal action of narcotics. Science 1976;192:1357–8.

Yoshimura M, North RA. Substancia gelatinosa neurons hyperpolarized in vitro by enkephalin. Nature 1983;305:529–30.

Zimmermann M. Ethical guidelines for investigations on experimental pain in conscious animals. Pain 1983;16:109–10.