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Evaluation of the interaction between acemetacin and opioids on the hargreaves model of thermal hyperalgesia

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Abstract

It has been shown that the association of opioids analgesic agents with non-steroidal anti-inflammatory drugs (NSAIDs) can increase their antinociceptive activity, allowing the use of lower doses and thus limiting side effects. Therefore, the goal of the present study was to examine the possible pharmacological interaction between acemetacin and two opioids in the Hargreaves model of thermal hyperalgesia in the mouse. Acemetacin, codeine, nalbuphine or fixed-dose ratios acemetacin–codeine and acemetacin–nalbuphine combinations were administrated systemically to mice and the antihyperalgesic effect was evaluated using the thermal hyperalgesia test. All treatments produced a dose-dependent antihyperalgesic effect. ED_{40} values were estimated for all the treatments and an isobologram was constructed. The derived theoretical ED_{40} for the acemetacin–codeine and acemetacin–nalbuphine combinations were 55.9±4.9 mg/kg and 40.3±3.8 mg/kg, respectively, being significantly higher than the actually observed experimental ED_{40}, 14.5±1.7 mg/kg and 12.7±2.2 mg/kg, respectively. These results correspond to synergistic interactions between acemetacin and opioids on the Hargreaves model of thermal hyperalgesia. Highest doses of the individual drugs or the combinations did not affect motor coordination in the balancing test on a rota-rod. Data suggest that low doses of the acemetacin–opioids combination can interact synergistically at systemic level and therefore this drugs association may represent a therapeutic advantage for the clinical treatment of inflammatory pain.

Keywords: Acemetacin; Codeine; Nalbuphine; Synergism; Thermal hyperalgesia; Mouse

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications in the world. NSAIDs provide effective management of pain and inflammation, but a mayor factor limiting their use is gastrointestinal damage. It has been established that as many as 2% to 4% of patients who regularly take NSAIDs may have a serious gastrointestinal side effect such as perforation, ulceration, or bleeding during long-term therapy (Wolfe et al., 1999; Fiorucci et al., 2001). The discovery of cyclooxygenase (COX)-2 has provided the rationale for the development of a new class of NSAIDs, the selective COX-2 inhibitors, with the aim of reducing the gastrointestinal toxicity associated with the administration of NSAIDs by virtue of COX-1 sparing (Hawkey, 1999; Wolfe et al., 1999; Fiorucci et al., 2001; Bombardier, 2002). However, selective COX-2 inhibitors have not fulfilled all the expectations of security. In this respect, some COX-2-selective NSAIDs are associated with an increased risk of serious adverse cardiovascular events compared to placebo, and valdecoxib was associated with an increased rate of serious and potentially life-threatening skin reactions (Mukherjee et al., 2001; Bresalier et al., 2005; Talhari et al., 2005). Therefore, physicians are eager to find a drug with fewer side effects for patients. Preferably, the ideal drug would be efficacious, not expensive and with few untoward side effects, making easier the

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decision to prescribe. For this reason, it is important to continue the investigation of either new or old NSAIDs that show a profile of suitable analgesic activity with a greater index of gastric and cardiovascular security.

Acemetacin is a glycolic acid of the NSAID indomethacin, characterized as a weak acid with potent anti-inflammatory, analgesic and antipyretic effects equivalent to those seen with indomethacin at an equimolar dose, however, gastric mucosa impairment caused by this drug is less than the one reported with indomethacin (Muller et al., 1986; Tavares and Bennett, 1993; Bori-Segura et al., 2002). In the same way, a prospective study that compared acemetacin with the COX-2-selective inhibitor celecoxib in the treatment of osteoarthritis of the knee joint revealed similar rates of efficacy and tolerability (Leeb et al., 2004). Taken together, these findings suggest that acemetacin is a good therapeutic option for those patients with antecedents of cardiovascular or gastrointestinal risk.

Opioids are some of the most efficacious analgesics used in humans (Cherny, 1996). Opioid analgesics are functionally classified as full agonists, partial agonists or mixed agonist-antagonists (Picker and Dykstra, 1989; Cherny, 1996). Opioids produce analgesia by binding to specific receptors (μ, δ, and κ) both within and outside the central nervous system (Simon, 1986; Dionne et al., 2001; Mignat et al., 1995). Codeine is an old drug that still enjoys widespread clinical use. It has been demonstrated that codeine is the one of most widely prescribed opioid analgesic in anesthetic practice (Stoneham and Walters, 1995; de Lima et al., 1996). Likewise, codeine causes low incidence of opioid-related side effects in younger age groups including neonates (Semple et al., 1999). Another effective analgesic is the opioid agonist-antagonist nalbuphine. It has been reported that the nalbuphine analgesic potency is essentially equivalent to that of morphine on a milligram basis (Culebras et al., 2000). Its main advantages over morphine are a ceiling effect on respiratory depression, low tolerance liability and a lack of significant withdrawal symptoms (Schmidt et al., 1985; Cohen et al., 1993; Chmielnicki, 1993).

NSAIDs, such as acemetacin, are indicated for the treatment of mild to moderate pain, whereas opioids such as codeine or nalbuphine are indicated for the treatment of moderate to severe pain. Opioids and NSAIDs are the most commonly used analgesics and their interaction is often favorable as it allows a reduction in opioid dosing (Strobel, 1992; Fletcher et al., 1997; Goldstein, 2002; Litkowski et al., 2005) and leading to a decrease in the incidence and intensity of side effects, such as gastrointestinal and respiratory alterations (Curatolo and Sveticic, 2002; Goldstein, 2002). Hence, the purpose of the present study was to characterize the antihyperalgesic effect of the systemic administration of the acemetacin–codeine and acemetacin–nalbuphine combinations in the Hargreaves model of thermal hyperalgesia in the mouse.

2. Material and methods

2.1. Animals

Balb/c male mice (weight range, 18–28 g) from our own breeding facilities were used in this study. Animals had free access to food and drinking water before experiments. Efforts were made to minimize animal suffering and to reduce number of animals used. Mice were used once only. At the end of the experiments, mice were sacrificed in a CO_2 chamber. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983) and the protocol was approved by the Institutional Animal Care and Use Committee.

2.2. Evaluation of thermal antihyperalgesia

Antihyperalgesia was assessed by the Hargreaves model of thermal hyperalgesia (Hargreaves et al., 1988). A plantar test (Ugo Basile apparatus) was used to measure the withdrawal latencies of the hind paws from a radiant heat stimulus. Mice were manually restrained and no pre-experiment habituation to the test environment was carried out (Menendez et al., 2002). The thermal nociceptive stimulus originated from a high intensity projector lamp bulb (infra-red intensity: 217 mW/cm^2) was manually manipulated and positioned under each footpad before and after the intraplantar injection of saline into the left hind paw or carrageenan (25 μl; 2%) into the right hind paw. A timer was automatically actuated with the light source, and the paw withdrawal latencies (PWLs) measured was defined as the time required for the paw to show an abrupt withdrawal. A cut-off time of 22 s was used to prevent tissue damage. Measurements of PWLs were made before and 1, 2, 3, 4, 5 and 6 h after saline or carrageenan injection.

2.3. Drugs

Acemetacin was purchased from Sigma (St. Louis, MO, USA). Carrageenan (Type IV, Lambda) was purchased from Research Biochemical International (Natick, MA, USA). Codeine phosphate was kindly supplied by Novartis Farmacéutica (Mexico). Codeine and nalbuphine hydrochloride (10 mg/ml ampoules, Bufigen®; Laboratorios Pisa, Mexico) were dissolved and diluted in 0.9% saline solution, respectively. Carrageenan was dissolved in 0.9% saline solution. Acemetacin was dissolved in Tween 20 and buffer solution (sodium hydroxide and monobasic potassium phosphate).

2.4. Study design

In order to assess the antihyperalgesic effect, thirty min before the carrageenan injection, animals were pre-treated with intraperitoneal (i.p.) and subcutaneous (s.c.) administrations of vehicles or increasing doses of acemetacin (10–56 mg/kg, i.p.), codeine (10–56 mg/kg, s.c.), nalbuphine (5.6–30 mg/kg, s.c.) or the acemetacin (i.p.)–codeine (s.c.) (6.99–55.9 mg/kg) and acemetacin (i.p.)–nalbuphine (s.c.) (5.04–40.35 mg/kg) combinations. The injection volumes were 100 μl. Mice in all groups were evaluated with regard to motor function changes induced by the treatments. Independent groups of mice were examined for coordination motor in a rotating horizontal rod (Ugo Basile apparatus) before and after receiving the highest doses of acemetacin, nalbuphine, codeine, acemetacin–opioids combinations or vehicles. The mice were trained twice upon a

cylinder rotating at a speed of 17 rpm at intervals of about 10 min after the final training (Vaz et al., 1996; Jones et al., 2005). Test compounds were injected 1 h after the final training. The experiment was carried out at 1, 4 and 6 h after injection. The mice were balanced on the rod for a maximum of 60 s. All observations were carried out by a blinded investigator.

2.5. Data analysis

Results are presented as mean±SEM for 8–12 animals per group. Time-courses of antihyperalgesic response of individual drugs and the combinations were constructed by plotting the PWLs as a function of time. The areas under the PWLs against time curves (AUC) were calculated by the trapezoidal rule. AUC was calculated and percent of antihyperalgesia was calculated according to the following equation: percent of antihyperalgesia $=[(AUC_{post\ compound}-AUC_{vehicle})/AUC_{vehicle}]\times 100$.

The dose–response curves were constructed and the experimental points fitted using least-squares linear regression. ED_{40}±standard error (SEM) was calculated according to Tallarida (2000).

It has been previously demonstrated that, for evaluation of the interaction between analgesic drugs, isobolographic analysis is a convenient tool (Tallarida, 2000). Therefore, in the present study, we used such technique to determine the nature of drug interaction between acemetacin and opioids. 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 thermal hyperalgesia, it appeared that acemetacin was unable to achieve a 50% response, and thus the calculation of ED_{50} was not feasible. Therefore, we estimated the ED_{40} instead of ED_{50} (Tallarida, 1992; Jiménez-Andrade et al., 2003). Subsequently, a dose–response curve was obtained by concurrent delivery of two drugs in a fixed-ratio, based on the ED_{40} values of each individual agent. To construct these curves, groups of animals

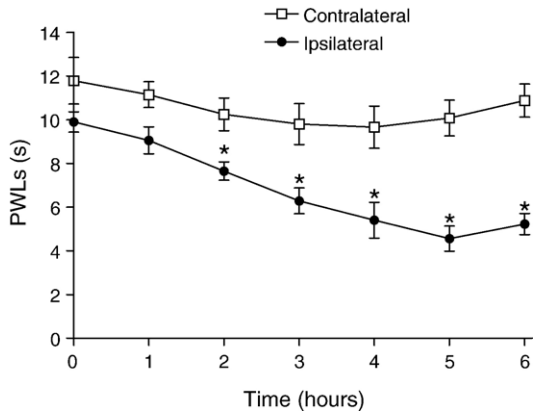

Fig. 1. Time course of paw withdrawal latencies (PWLs) induced by exposure to radiant heat in mice injected with saline into the left hind paw (contralateral) or carrageenan (2%, 25 μl) into the right hind paw (ipsilateral). Data are the means± SEM for 8–12 animals. *Significantly different from saline ($p<0.05$), as determined by analysis of variance followed by Dunnet's test.

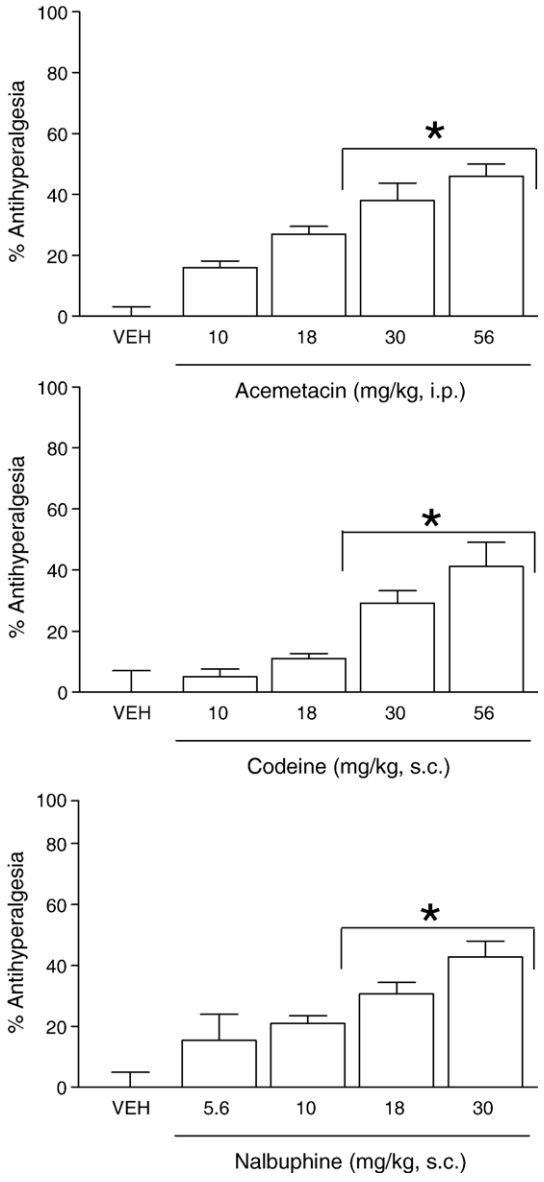

Fig. 2. Effect of intraperitoneal administration of acemetacin (10–56 mg/kg, i.p.) and of subcutaneous administration of codeine (10–56 mg/kg, s.c.) or nalbuphine (5.6–30 mg/kg, s.c.) in carrageenan-induced thermal hyperalgesia. Mice were pretreated with vehicle, codeine or nalbuphine 30 min before carrageenan injection. Data are expressed as the percentage of antihyperalgesia. Bars are the means±SEM for 8 to 12 animals. *Significantly different from vehicle ($p<0.05$), as determined by analysis of variance followed by Dunnet's test.

received one of the following doses of the combination: (acemetacin ED_{40}+opiate ED_{40})/2; acemetacin ED_{40}+opiate ED_{40})/4; acemetacin ED_{40}+opiate ED_{40})/8; acemetacin ED_{40}+opiate ED_{40})/16. The experimental ED_{40} values for the combinations were calculated from these curves.

The theoretical additive ED_{40} was estimated from the dose–response curves of each drug administered individually, i.e. considering that the observed effect with the combination is the outcome of the sum of the effects of each the individual drug. This theoretical ED_{40} value is then compared with the experimentally derived ED_{40} to determine if there is a statistically significant difference (Tallarida, 2002; Tallarida et al., 1999). The theoretical and experimental ED_{40} values of the studied combinations were

also contrasted by calculating the interaction index (γ) as it follows: $\gamma = ED_{40}$ of combination (experimental)/ED_{40} of combination (theoretical).

An interaction index not significantly different from unity corresponds to an additive interaction whereas values higher and lower than unity imply an antagonistic and synergistic interaction, respectively (Tallarida, 2002; Jiménez-Andrade et al., 2003).

2.6. 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_{40} and the experimentally derived ED_{40} values was evaluated using Student's *t* test (Tallarida, 2000). An experimental ED_{40} significantly lower than the theoretical additive ED_{40} was considered to indicate a synergistic interaction between acemetacin and opiate. Statistical significance was considered to be achieved when $p < 0.05$.

3. Results

3.1. Systemic antihyperalgesic effect of acemetacin, codeine, nalbuphine and the combinations

Our data indicate, first of all that intraplantar carrageenan (25 µl, 2%) into the right hind paw, but not saline in the contralateral paw, produced a time-dependent thermal hyperalgesic effect (Fig. 1). A significant reduction in PWLs was observed at 2 h post carrageenan which was evaluated by 6 h (Fig. 1). Besides, the administration of acemetacin, codeine, nalbuphine or the acemetacin–codeine and acemetacin–nalbuphine combinations, but not vehicles, produced a dose-dependent reduction in the hyperalgesic effect induced by carrageenan ($p < 0.05$, Figs. 2 and 3).

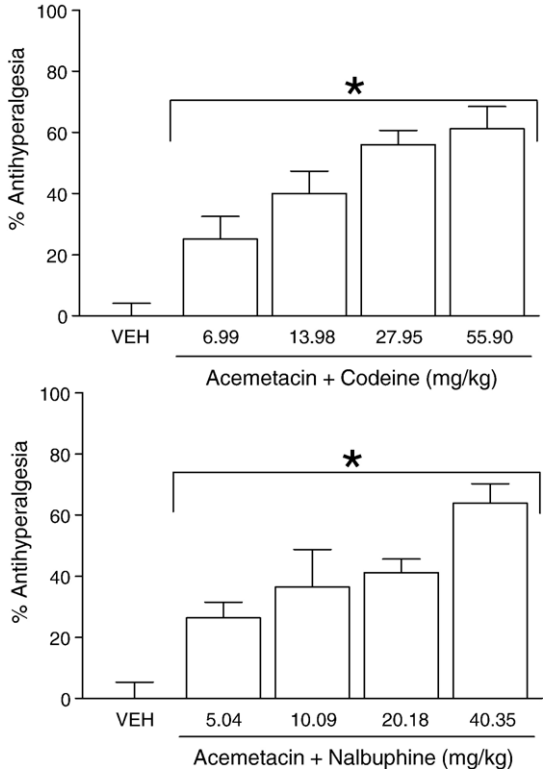

Fig. 3. Effect of the acemetacin–opioid combinations in carrageenan-induced thermal hyperalgesia. Mice were pretreated with vehicle or acemetacin–codeine or acemetacin–nalbuphine 30 min before carrageenan injection. Data are expressed as the percentage of antihyperalgesia. Bars are the means ± SEM for 8 to 12 animals. *Significantly different from vehicle ($p < 0.05$), as determined by analysis of variance followed by Dunnet's test.

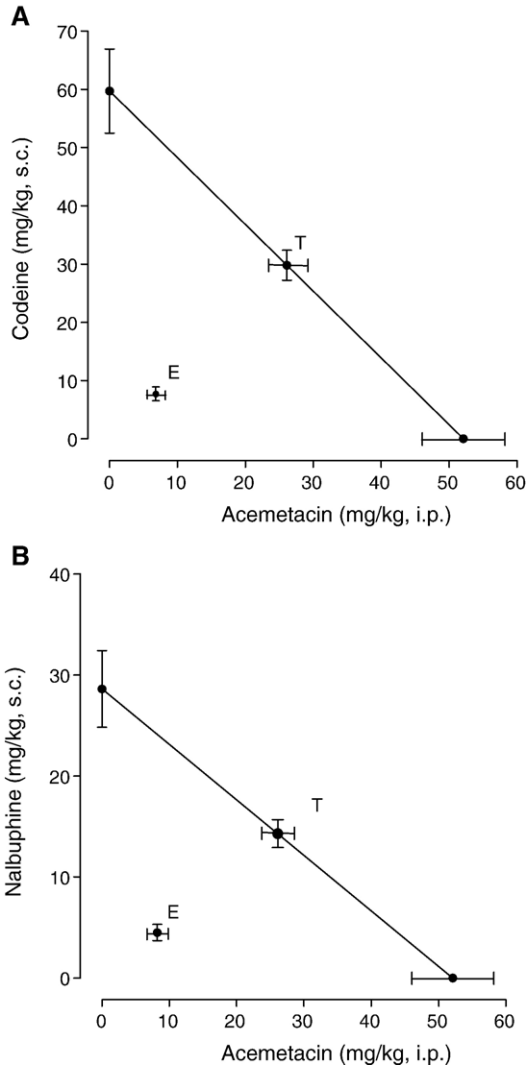

Fig. 4. Isobolograms showing the systemic interaction between acemetacin and opioids on the Hargreaves model of thermal hyperalgesia. A. Acemetacin–codeine combination. B. Acemetacinnalbuphine combination. The oblique lines between the *x* and *y* axis are the theoretical additive lines. The points in the middle of these lines, indicated by "T", are the theoretical additive points calculated from the individual drug ED_{40} values. The experimental points indicated by "E", are the actually observed ED_{40} values with the combinations. Horizontal and vertical bars indicate SEM.

3.2. Interaction of acemetacin and opioids after systemic administration

The ED_{40} values for acemetacin, codeine and nalbuphine on the thermal hyperalgesia test were 52.1 ± 6.7 mg/kg, 59.7 ± 7.2 mg/kg and 28.6 ± 3.8 mg/kg, respectively. Fixed-dose ratio

combinations were prepared, as described above, and assayed in order to construct the dose–response curves for the combinations and calculate the corresponding experimental ED_{40}, which were 14.5±1.7 mg/kg and 12.7±2.2 mg/kg for acemetacin–codeine and acemetacin–nalbuphine combinations, respectively. These values were significantly lower ($p<0.05$) than the theoretical ED_{40} expected for a purely additive interaction, which were 55.9±4.9 mg/kg and 40.3±3.8 mg/kg for the acemetacin–codeine and acemetacin–nalbuphine combinations, respectively, as it can be clearly appreciated in Fig. 4, the experimental ED_{40} values being located below the additive dose line. Furthermore, the interaction indexes (γ) were 0.26± 0.04 and 0.31±0.06 for acemetacin–codeine and acemetacin–nalbuphine combinations, respectively, being statistically different from unity. Data thus strongly suggest that the interactions between the antihyperalgesic actions of acemetacin and opioids at systemic level are synergistic, the resulting effects being about three times higher than that expected by the sum of the effects of the individual components. Finally, at the maximal dose of each drug or combination used in our study did not significantly affect the motor response on the rota-rod test ($p>0.05$). Control response in the rota-rod test during 1, 4 and 6 h were 60±0 s, 60±0 s and 58.6±1.4 s, respectively. Codeine (56 mg/kg, i.p.) responses during 1, 4 and 6 h were 60±0 s, 60±0 s and 60±0 s, respectively. Nalbuphine (30 mg/kg, s.c.) responses during 1, 4 and 6 h were 60±0 s, 57.6±2.4 s and 58.4±1.6 s, respectively. Likewise, the responses in the rota-rod test during 1, 4 and 6 h were 60±0 s, 58.1±1.9 s and 60±0 s, respectively, in the presence of acemetacin–codeine (55.9 mg/kg) and of 58±1.4 s, 58.8±1.3 s and 60±0 s, respectively, in the presence of acemetacin–nalbuphine (40.35 mg/kg).

4. Discussion

4.1. Antihyperalgesic effect induced by acemetacin

In several fields, non-steroidal anti-inflammatory analgesic agents are widely used as one of medicinal treatments for pain or inflammation. Acemetacin is a NSAID which has a chemical structure similar to that of indomethacin. The findings of the similar potencies of acemetacin and indomethacin on leukocyte COX and the lower potency of acemetacin on the gastric mucosa COX are consistent with an effective analgesic and anti-inflammatory activity of acemetacin coupled with better gastric tolerance than to indomethacin (Badia-Flores and Muñoz Barradas, 1980; Muller et al., 1986; Tavares and Bennett, 1993; Bori-Segura et al., 2002). In the present study, systemic administration of acemetacin was able to decrease the hyperalgesic effect induce by carrageenan in the mouse. Therefore, it is likely that the antihyperalgesic effect observed in our study could result from inhibition of prostaglandins release at central and peripheral levels evoked by tissue injury. However, it is accepted that some NSAIDs besides to produce *in vitro* and *in vivo* inhibition of COX, have additional mechanisms of action (Jacobi and Dell, 1980; Voilley et al., 2001; Ortiz et al., 2002, 2003, 2005a; Guevara-López, 2004). At this respect, it has been demonstrated that acemetacin is able to inhibit the locomotion of neutrophils and the histamine release of isolated mast cells (Jacobi and Dell, 1980; Guevara-López, 2004). Consequently, it is possible that the critical role played by neutrophils, prostaglandins and histamine in the thermal hyperalgesic response might be influenced by acemetacin, which would explain, at least in part, its anti-inflammatory and antihyperalgesic properties.

4.2. Antihyperalgesic effect induced by opioids

Although it has been demonstrated that the opioid analgesic codeine produces peripheral antinociception by activation of the opioid receptor-nitric oxide-cyclic GMP-K^+ channels pathway (Ortiz et al., 2005b), there have been many reports suggesting that the systemic analgesic effect of codeine is either wholly or mostly dependent on its metabolism to morphine, codeine-6-glucuronide and norcodeine (Chen et al., 1991; Yue et al., 1991; Cleary et al., 1994). On the other hand, the opioid agonist-antagonist analgesic nalbuphine binds to mu and kappa receptors, acting as an agonist at kappa receptors but as an antagonist at the mu receptor (Schmidt et al., 1985; Hoskin and Hanks, 1991). In the present work, systemic administration of either codeine or nalbuphine caused dose-dependent increases in inflamed paw withdrawal latencies to thermal stimulation. These results obtained in the animal experiments agree with previous researches that have reported on increased paw withdrawal latencies to heat and mechanical stimuli after parenteral administration of opioids in animals (Lahdesmaki et al., 2003; Hau et al., 2004; Bileviciute-Ljungar et al., 2006).

4.3. Antihyperalgesic effect induced by the combinations

In the current work, isobolographic analysis demonstrated a significant synergistic interaction between acemetacin and codeine at systemic level. These results confirm previous experiments showing that co-administration of NSAIDs significantly increases the effect of codeine (Cooper et al., 1982; Forbes et al., 1986; Strobel, 1992; de Craen et al., 1996; Jiménez-Andrade et al., 2003). Codeine may decrease the presynaptic glutamate release at spinal level by Ca^{2+} channels inhibition (Cadet, 2004) and activation of the opioid receptor-nitric oxide-cyclic GMP-K^+ channel pathway (Ortiz et al., 2005b) in presynaptic neurons. Acemetacin may synergize with these effects through its ability to inhibit COX and accordingly block the synthesis and release of prostaglandins (Jacobi and Dell, 1980) and inhibition of neutrophils and histamine release (Jacobi and Dell, 1980; Guevara-López, 2004), which would reduce the excitability of presynaptic neurons and postsynaptic dorsal horn neurons.

In the present study, the nalbuphine–acemetacin association provided a significantly synergistic interaction. This last data is supported by studies that have demonstrated that the mixture of nalbuphine with NSAIDs appears to be a therapeutically useful combination (Jain et al., 1986; Monrigal et al., 1994). Functional interaction may result from distinct drug effects at separate anatomic sites that may act independently and together to inhibit general nociceptive processing. In this respect, it has

been demonstrated that nalbuphine elicits analgesia through a complex interaction of supraspinal and spinal kappa receptor mechanisms (Parsons et al., 1989; Pick et al., 1992). Recently, our group has found that nalbuphine is able to activate ATP-sensitive K^+ channels, in order to produce its peripheral antinociceptive effect on the rat formalin test (Ortiz and Castañeda-Hernández, 2006). Therefore, it is possible that a decrease in excitatory neurotransmission with nalbuphine is produced at three levels: peripheral, spinal and supraspinal. Thus, these nalbuphine effects and accompanied by the acemetacin actions may lead to synergism.

Previous studies have shown that the concurrent use of opioids and NSAIDs produces increased antinociception or a reduction in the requirements of opioid agents (Strobel, 1992; Fletcher et al., 1997; Tallarida et al., 1999; Reuben and Connelly, 2000; Goldstein, 2002; Litkowski et al., 2005; Bourlert, 2005; Rahimi et al., 2006). Clinical outcomes of the acemetacin–opioids co-administration could include greater analgesia and attenuation of opioid-induced adverse reactions such as nausea, vomiting, constipation, sedation and respiratory depression. On the other hand, acemetacin has shown suitable effectiveness and excellent tolerability in different human populations studied (Bori-Segura et al., 2002; Guevara-López, 2004). For this reason, the synergism observed suggests that this combination could have better gastrointestinal and renal side-effects profile than acemetacin alone. The efficacy and benefits of this combination in clinical situations await supplementary validation.

It has been demonstrated that for evaluation of the interaction between analgesic drugs isobolographic analysis is a convenient tool. For analgesic compounds, for example, one might be an opiate and thus have a central action, whereas the other could be a NSAID with periphery effect. What is important is that each drug demonstrates a dose-dependent common effect which can be quantified when the drugs are tested individually or in combination. The drugs may differ markedly in potency and/or efficacy (Ossipov et al., 1990; Tallarida, 1992, 2000; Tallarida et al., 1999). Thus, from the dose–response curves of each individual agent, the dose resulting in 50% of the effect (ED_{50}) can be determined. Nevertheless, considering a maximal effect of 100% as the total suppression of thermal hyperalgesia, it is important to point out that in the present study the dose of 56 mg/kg or higher doses of acemetacin were unable to achieve a 50% response, and thus the calculation of ED_{50} was not feasible. Therefore, we estimated the ED_{40} instead of ED_{50} for all the drugs. However, the election of an ED minor or different than ED_{50} has shown to be a convenient tool for isobolographic analysis (Ossipov et al., 1990; Tallarida, 1992; Tallarida et al., 1999; Jiménez-Andrade et al., 2003; Granados-Soto and Argüelles, 2005; Picazo et al., 2006; Bhat et al., 2007). A second limitation was that our experimental design generated isobols for only one fixed ratio mixture (1:1). Nevertheless, it has been mentioned by other authors that the fixed-ratio combinations permit easy dose adjustments, have direct application to drug development and are amenable to statistical analysis. Further, the findings that synergism occurs at one ratio may aid in uncovering its mechanism. Therefore, we thought that the synergism observed in our study with the acemetacin–opioids combinations could serve as one first track to make more studies in the future. On the other hand, there are several studies that have demonstrated the utility of synergism at one ratio (Ossipov et al., 1990; Tallarida, 1992; Tallarida et al., 1999; Yoon and Yaksh, 1999; Raffa et al., 2000; Jiménez-Andrade et al., 2003; Czuczwar et al., 2003; Granados-Soto and Argüelles, 2005; Picazo et al., 2006; Bhat et al., 2007).

Finally, the administrations of the highest doses of the drugs used in our study did not significantly affect the motor response on the rota-rod test. This was expected as it has been previously demonstrated that systemic injections of codeine, nalbuphine or acemetacin did not result in any significant alteration of motor activity (Jacobi and Dell, 1980; Pick et al., 1992; Jiménez-Andrade et al., 2003; Guevara-López, 2004).

In summary, acemetacin and opioids (codeine and nalbuphine) combinations produced an antihyperalgesic effect on the Hargreaves model of thermal hyperalgesia. Data suggest that low doses of the acemetacin–opioids combination can interact synergically at systemic level and therefore these drug associations may represent a therapeutic advantage for the clinical treatment of inflammatory pain. Therefore, clinical studies assessing the therapeutic potential of these combinations are encouraged.

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