Pharmacological Reports
2011, 63, 433–440
ISSN 1734-1140

Synergism between dexketoprofen and meloxicam in an orofacial formalin test was not modified by opioid antagonists

Claudia Gonzalez[1], Carlos Zegpi[1], Viviana Noriega[2], Juan C. Prieto[1,3], Hugo F. Miranda[1,2]

[1]School of Medicine, Pharmacology Program, ICBM, Faculty of Medicine, University of Chile, Independencia 1027, Clasificador 70.000, Santiago 7, Chile

[2]Faculty of Medicine, School of Pharmacy, Andrés Bello University, Av. República 252, Santiago, Chile

[3]Cardiovascular Department, Clinical Hospital, University of Chile, Santos Dumont 999, Independencia 7, Santiago, Chile

Correspondence: Hugo F. Miranda, e-mail: hmiranda@med.uchile.cl

Abstract:
Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used drugs for the management of acute and chronic pain. The role of the opioid system in the synergism between NSAIDs is not well characterized. Mice were injected with a 5% formalin solution (20 μl) into the upper right lip to perform an orofacial formalin test. The isobolographic method was used to determine the interaction between dexketoprofen, which is the (S)-(+) enantiomer of ketoprofen, and meloxicam co-administration. Additionally, the non-selective, opioid antagonist naltrexone, the selective δ opioid receptor (DOP) antagonist naltrindole and the selective κ opioid receptor (KOP) antagonist norbinaltorphimine were used to assess the opioid effects on this interaction. Intraperitoneal administration of dexketoprofen or meloxicam induced dose-dependent antinociception with different phase I and phase II potencies in the orofacial formalin test. Meloxicam displayed similar potencies (ED_{50}) in phase I (7.20 mg/kg) and phase II (8.60 mg/kg). Dexketoprofen was more potent in phase I (19.96 mg/kg) than in phase II (50.90 mg/kg). The interactions between dexketoprofen and meloxicam were synergistic in both phases. This was determined based on the fixed ratios (1:1) of their ED_{50} values, which were determined by isobolographic analysis. Furthermore, this antinociceptive activity does not seem to be modulated by opioid receptor blockers because they did not induce changes in the nature of this interaction. This finding may be relevant with regards to NSAID multi-modal analgesia where an opioid antagonist must be used.

Key words:
algesiometric tests, antinociception, isobolographic analysis, synergism

Introduction

Exaggerated or diminished effects will sometimes occur when drugs with similar effects are used concurrently [24]. In certain cases, co-administering antinociceptive agents results in synergistic effects; therefore, the doses of each drug can be reduced [18].

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used classes of drugs for the management of acute and chronic pain. They pre-

vent the development of inflammation and produce their analgesic effects by blocking the synthesis of prostaglandins (PGs) in the periphery by inhibiting cyclooxygenase enzymes. These enzymes catalyze PG synthesis from arachidonic acid. Three isoforms of cyclooxygenase (COX) have been identified: COX-1 (expressed constitutively throughout the body), COX-2 (expressed predominantly in inflammatory processes) and COX-3 (a recently identified isoform that is mainly located in the human cerebral cortex). NSAIDs provide excellent analgesia for mild to moderate pain. They are particularly useful in the initial management of pain with an inflammatory component [21].

Opioids are the most effective drugs used in treating severe pain, and they exert their actions by interfering with pain in the central nervous system [11]. However, unwanted side effects may seriously limit their clinical use. Combinations of opioids and COX-2 inhibitors have shown synergistic interactions and are in clinical use for postoperative pain [12, 14]. To date, four opioid receptors have been cloned: MOP (μ for morphine), KOP (for ketocyclazocine), DOP (δ for deferens; it was first identified in mouse vas deferens) and NOP (for nociceptin) [28]. However, there is a disparity between the existences of only four opioid receptor genes and the substantial pharmacological evidence for additional opioid receptor phenotypes.

Few reports have studied the synergy between COX-1 and COX-2 inhibitors using isobolographic analysis in acute and inflammatory orofacial pain. The purpose of the present study was to assess the interaction between the (S)-(+) enantiomer of racemic ketoprofen (dexketoprofen), which is a COX-1 inhibitor [15] that inhibits PG activity, and a selective COX-2 inhibitor (meloxicam) in a modified formalin orofacial model [19]. In addition, we assessed the effects of opioid receptors on this interaction.

Materials and Methods

Male CF-1 mice (35–40 days old, weighing 29 ± 1.5 g) were housed in a 12 h light-dark cycle at 22 ± 1°C, and they had free access to food and water. The animals were acclimatized to the laboratory environment for at least 2 h before the experiments began. Experiments were carried out in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals, and the Institutional Animal Care and Use Committee at the University of Chile (Santiago, Chile) approved all experimental procedures. Each animal was used only once and received only one dose of the drugs tested. All drugs were freshly prepared in normal saline and administered intraperitoneally (*ip*). All observations were performed by the authors in randomized and blinded manners. Control animals were given saline and were run interspersed concurrently with the drug-treated animals (at least two mice per group) to prevent the controls from being run in a single group at one time.

Orofacial formalin test

The method described by Luccarini et al. [13] was used for the orofacial formalin test with modifications. To perform the test, a 5% formalin solution (20 μl) was injected into the upper right lip of each mouse with a 27-gauge needle. In the preliminary experiments, different groups of mice were treated with different formalin concentrations (1, 2 or 5%) to establish the concentration-response relationships for both phases. Based on these results, we selected the 5% formalin dose because inhibition was easy to detect. After the formalin injection, mice were immediately returned to a glass observation chamber. The degree of pain intensity was assessed by the total time that the animal spent rubbing its lip with one of its extremities. Administration of the analgesics (or saline solution for the control group) and the opioid receptor blockers occurred 30 min and 1 h, respectively, before formalin administration. Two phases were distinguished during the assay. Phase I corresponded to the 5-min period starting immediately after formalin injection that represents tonic acute pain due to peripheral nociceptor sensitization. Phase II was recorded as the 10-min period starting 20 min after formalin injection and represents inflammatory pain. Drug effects were characterized after the administration of at least four doses in logarithmic increments. The maximum possible effect (MPE) was calculated as follows:

$$\% \text{ MPE} = 100 - [\text{post drug rubbing time/control rubbing time} \times 100]$$

The dose that produced 50% of the MPE (ED_{50}) was calculated from the linear regression analysis of the curve that was obtained by plotting the log dose *vs.* % MPE.

Protocol

Dose-response curves were obtained for dexketoprofen or meloxicam administration (*ip*) using at least six animals and at least four doses. Linear regression analysis was performed on the log dose-response curve. This analysis allowed for calculating the doses that produced 50% antinociception when each drug was administered alone. The ED_{50} values that were used in the orofacial formalin test were the equieffective doses used in the isobolographic analysis. This is because higher doses did not show increased effects without motor impairment [17, 18]. A similar dose-response curve was obtained and analyzed after the co-administration of dexketoprofen with each of the previously identified NSAIDs. These were administered in fixed-ratio (1:1) combinations based on mixtures that were 1/2, 1/4, 1/8, and 1/16 of their respective ED_{5o} values.

Isobolographic analysis

An isobolographic analysis was used to characterize the drug interactions, and its method has been described previously in detail [18]. An isobologram was constructed by plotting the ED_{50} of dexketoprofen on the abscissa and the ED_{50} of meloxicam on the ordinate to obtain the additivity line. For the drug mixture, the ED_{50} and its associated 95% confidence intervals were determined by linear regression analysis of the log dose-response curve (eight animals at each of at least four doses). Using a Student's *t*-test, the ED_{50} value was compared to a theoretical additive ED_{50} obtained from the calculation:

$$ED_{50}\ \text{add} = ED_{50}\ \text{meloxicam}/(P1 + R \times P2)$$

where R is the potency ratio of meloxicam alone to dexketoprofen alone, and P1 and P2 are the proportions of meloxicam and dexketoprofen in the total mixture, respectively. Fixed-ratio proportions were selected by first combining the ED_{50} value for each compound. Next, a dose-response curve was constructed where ED_{50} fractions (1/2, 1/4, 1/8 and 1/16) of dexketoprofen and meloxicam combinations were administered. Using the equation above, ED_{50} add was the total dose, and the ED_{50} add variance was calculated from the fraction of the ED_{50} values (i.e., 0.5) in the combinations as follows:

$$\text{Var}\ ED_{50}\ \text{add} = (0.5)^2\ \text{Var}\ ED_{50}\ \text{meloxicam} + (0.5)^2\ \text{Var}\ ED_{50}\ \text{dexketoprofen}$$

From these variances, confidence limits were calculated and resolved according to the ratio of the individual drugs in the combination. The ED_{50} for the drug combinations were obtained by linear regression analysis of the dose-response curves. Supra-additivity or synergistic effect is defined as the effect of a drug combination that is higher and statistically different (ED_{50} significantly lower) than the theoretically calculated equieffective drug combination with the same proportions. If the ED_{50} values are not statistically different, the effect of the combination is additive, and additivity means that each constituent contributes with its own potency to the total effect. The interaction index (I.I.) was calculated as the experimental ED_{50}/the theoretical ED_{50}. Values close to 1 show additive interactions. Values lower than 1 indicate supra-additive or synergistic interactions, and values higher than 1 correspond to sub-additive or antagonistic interactions [16, 18].

Drugs

All drugs were freshly dissolved in saline in a constant volume of 10 ml/kg and administered *ip*. Dexketoprofen and meloxicam were administered at doses between 1 and 300 mg/kg. The opioid antagonists' doses were adapted or modified from previously published studies that showed the pharmacological activity of each individual receptor subtype, and these doses were tested at the peak effect (30 min) [20, 22, 29, 31]. Dexketoprofen was a gift from Menarini Laboratories (Spain), meloxicam was purchase from Saval Laboratories, Chile and naltrexone hydrochloride, naltrindole hydrochloride and norbinaltorphimine dihydrochloride were purchased from Sigma Chemical Co. (USA).

Statistical analyses

Results are presented as the mean ± SEM or as ED_{50} values with 95% confidence limits (95% CL). Isobolographic calculations were performed with Pharm Tools Pro (version 1.1.27, The McCary Group Inc.) based on Tallarida [25]. Statistical analysis of the isobolograms was performed according to Tallarida [25] and differences between experimental and theoretical values were assessed by a Student's *t*-test for independent means; p values less than 0.05 ($p < 0.05$) were considered significant.

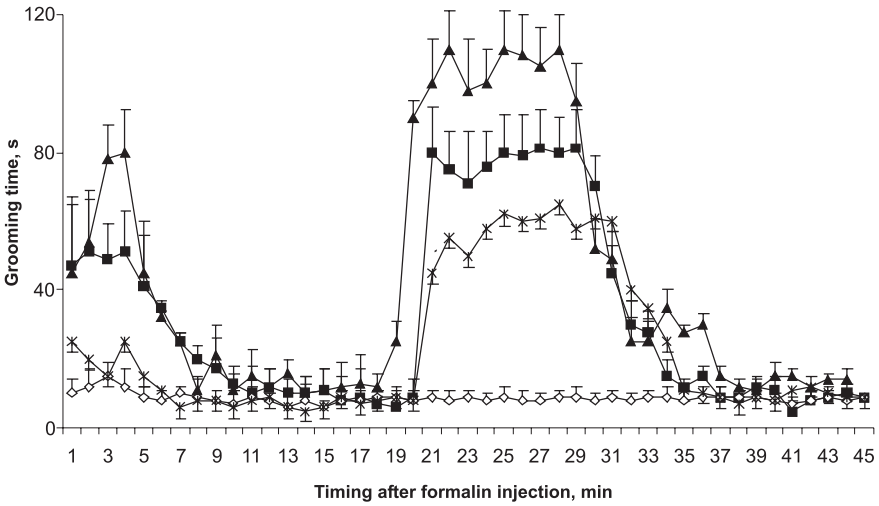

Fig. 1. Time course of the grooming activity in mice during the orofacial formalin test. Saline (◇), 1% formalin (✱), 2% formalin (■) and 5% formalin (▲). Each point represents the mean with SEM of at least 6 mice

Tab. 1. Dexketoprofen (DEX) and meloxicam (MELO) ED_{50} values and interaction indexes (I.I.) in phase I of the orofacial formalin test, and the effects of opioid blockers (naltrexone (NTX), naltrindole (NTI) and norbinaltorphimine (Nor-BNI)) on these values

Drugs	ED_{50}	I.I.
DEX	19.9 ± 2.02	
MELO	7.20 ± 1.45	
DEX + MELO theoretical	13.6 ± 0.04	
DEX + MELO experimental	5.61 ± 0.23*	0.413
DEX + MELO + NTX	3.36 ± 0.49*	0.247
DEX + MELO + NTI	5.33 ± 0.82*	0.437
DEX + MELO + Nor-BNI	3.20 ± 0.49*	0.259

* $p < 0.05$ *vs.* DEX + MELO theoretical

Tab. 2. Dexketoprofen (DEX) and meloxicam (MELO) ED_{50} values and interactions indexes (I.I.) in phase II of the orofacial formalin test, and the effects of opioid blockers (naltrexone (NTX), naltrindole (NTI) and norbinaltorphimine (Nor-BNI)) on these values

Drugs	$^{ED}_{50}$	I.I.
DEX	50.9 ± 7.14	
MELO	8.60 ± 0.80	
DEX + MELO theoretical	29.7 ± 0.06	
DEX + MELO experimental	4.36 ± 0.50*	0.147
DEX + MELO + NTX	6.88 ± 0.83*	0.298
DEX + MELO + NTI	5.02 ± 0.82*	0.174
DEX + MELO + Nor-BNI	5.10 ± 0.73*	0.171

* $p < 0.05$ with respect to DEX + MELO theoretical

Results

Nociceptive behavioral responses

The time course of the nociceptive responses to the modified orofacial formalin test is presented in Figure 1. The nociceptive responses presented with typical biphasic time courses of an early, short-lasting (5 min) first period of activity (Phase I), a 15-min quiescent period, and a second, prolonged (10 min) tonic phase (Phase II).

Antinociception induced by analgesic drugs

Administration of dexketoprofen or meloxicam induced dose-dependent antinociceptive activity with different potencies in Phase I and Phase II of the orofacial formalin test. The dose response curves for the different NSAIDs are presented in Figure 2. The phase I and in phase II dose response curves were statistically parallel, and the slopes for dexketoprofen and meloxicam were 38.48 ± 3.17 and 44.88 ± 2.61, respectively. Meloxicam displayed similar potencies in both phases; however, dexketoprofen was 3-fold more potent in phase I than in phase II (Tabs. 1 and 2).

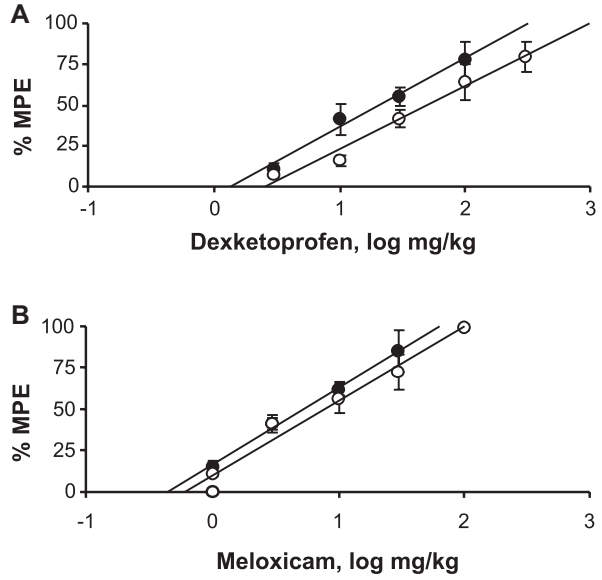

Fig. 2. Dose-response curves for the antinociceptive activities in mice that were induced by intraperitoneal administration of dexketoprofen (**A**) and meloxicam (**B**) in phase I (●) and phase II (○) of the orofacial formalin test. Each point is the mean ± SEM of 6–8 animals. MPE = maximum possible effect. Linear regressions for dexketoprofen: y = 42.638x + (–5.44) and y = 38.485x + (–15.683) for phase I and II, respectively. Linear regressions for meloxicam: y = 47.814x + (8.916) and y = 44.886x + (8.032) for phase I and II, respectively

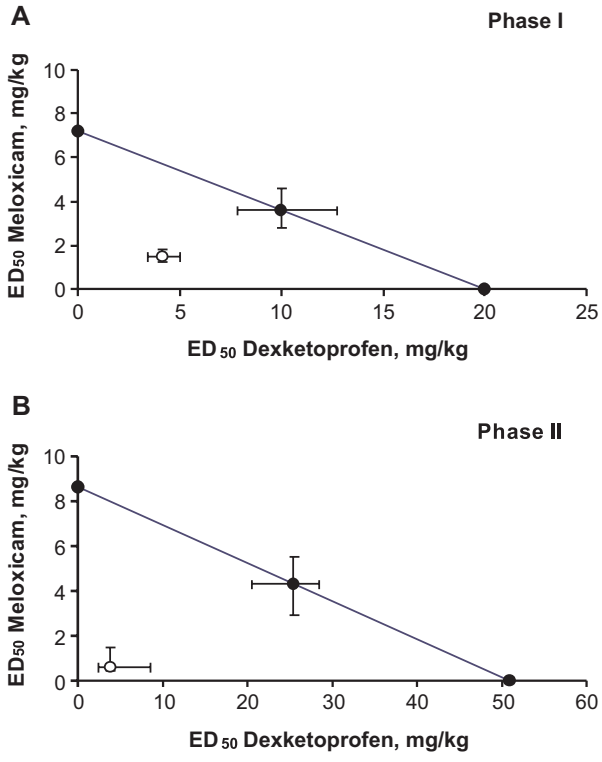

Fig. 3. Phase I (**A**) and phase II (**B**) isobolograms for the coadministration of dexketoprofen and meloxicam using the orofacial formalin test in mice. Theoretical ED_{50} value with 95% CL (●). Experimental ED_{50} value with 95% CL (○)

Interaction between dexketoprofen and meloxicam

Isobolographic analysis was used to calculate the interactions between dexketoprofen and meloxicam based on the fixed ratios (1:1) of their ED_{50} values. The theoretical additive ED_{50} values and the experimental ED_{50} values for the fixed ratio combinations are shown in Tables 1 and 2. Synergistic interactions between dexketoprofen with meloxicam are shown in Figure 3. The interaction index values of the different phase combinations are shown in Tables 1 and 2.

Effects of opioid antagonists on the interaction between dexketoprofen and meloxicam

The opioid antagonists naltrexone (0.1 mg/kg, *ip*), naltrindole (0.1 mg/kg, *ip*) and norbinaltorphimine (0.1 mg/kg, *ip*) did not possess intrinsic effects in the orofacial formalin test. They were also not able to significantly modify the magnitude of dexketoprofen's and meloxicam's antinociceptive effects (data not shown). Pre-treating animals with these opioid receptor blockers did not induce any significant changes in the antinociceptive activity of dexketoprofen and meloxicam in either phase I or phase II ($p < 0.05$) (Fig. 4) Corresponding I.I. values are summarized in Tables 1 and 2.

Discussion

The *ip* administration of either dexketoprofen or meloxicam to mice induced dose-dependent antinociceptive activity in a modified orofacial formalin test. These results are in agreement with the antinociceptive activities of these NSAIDs in other algesiometric assays, such as acetic acid writhing, formalin hind paw, tail-flick, and hot plate [18–20, 25]. In this study, a 5% formalin concentration was used because it was easy to detect the inhibitory treatment in both phases of the assay.

The parallel dose-response curves that were obtained with dexketoprofen and meloxicam in both phases are consistent with similar mechanisms of action, such as inhibiting COXs [9]. It is well known that dexketoprofen acts principally through inhibiting COX-1 [15], and meloxicam is a selective and specific COX-2 inhibitor [4, 10].

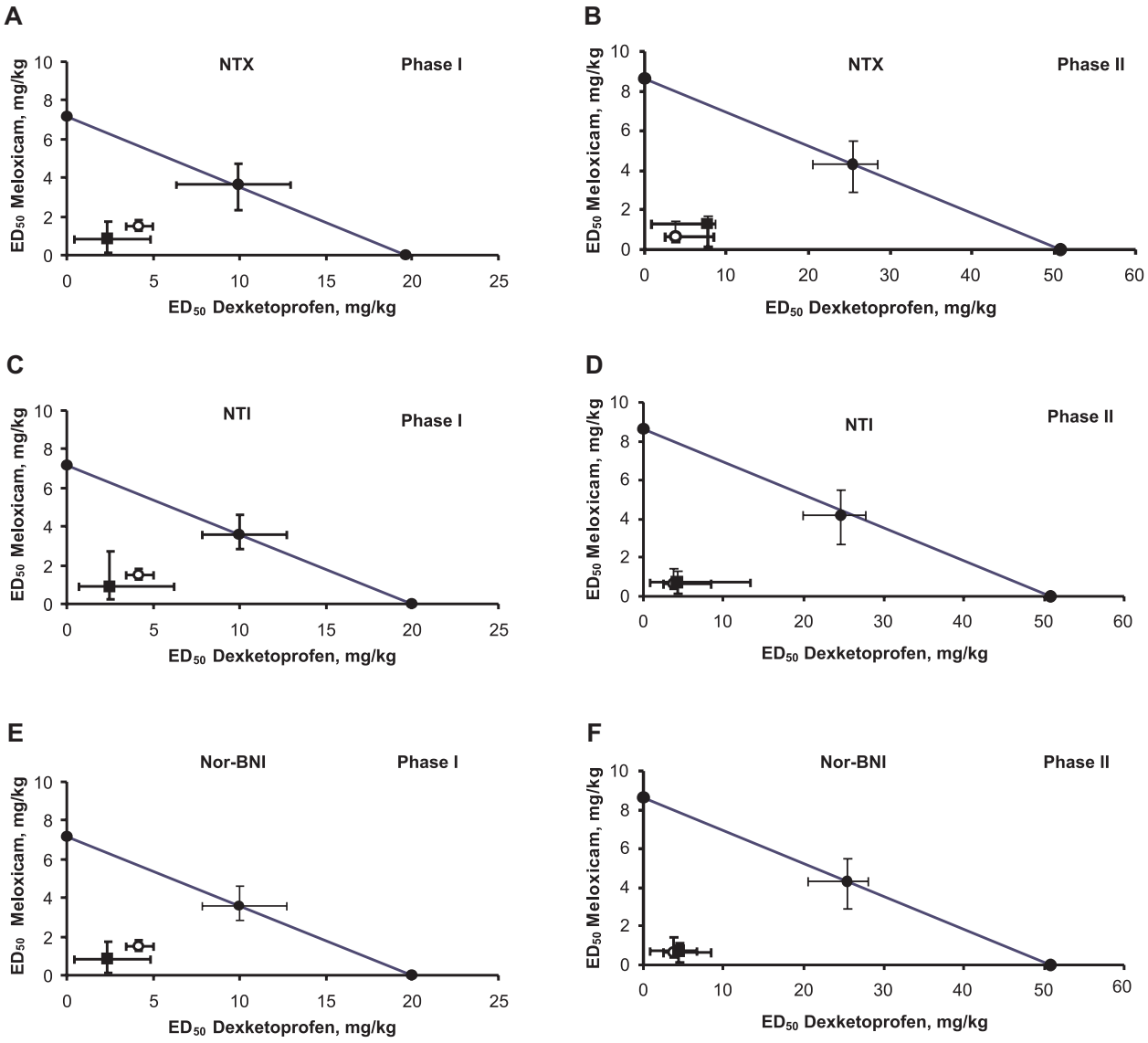

Fig. 4. Phase I and phase II isobolograms for the co-administration of dexketoprofen and meloxicam using the orofacial formalin test in mice. Theoretical ED_{50} with 95% CL (●). Experimental ED_{50} with 95% CL (O). Experimental value obtained after pretreatment with naltrexone (NTX, 0.1 mg/kg; **A** and **B**), naltrindole (NTI, 0.1 mg/kg; **C** and **D**) and norbinaltorphimine (NOR- BNI, 0.1 mg/kg; **E** and **F**) (■)

In this study, an isobolographic analysis was used to determine that the interaction between dexketoprofen and meloxicam was synergistic during the first and second phases of the assay. This result can be explained by the different mechanisms of action that each NSAID has towards the COXs [23]. The precise mechanisms of pain control in orofacial pain are largely unknown; however, the trigeminal system appears to be engaged [19]. The mechanisms responsible for the synergistic, antinociceptive activities between NSAIDs are also not clear. There are a number of possible mechanisms to explain the synergistic interactions among analgesic drugs, and they involve virtually all levels of cell function [2]. For example, dexketoprofen might enhance the affinity of meloxicam for its respective COX, decrease the rate of elimination of the NSAID, and enhance G-protein activation to consequently increase NSAID activity. Furthermore, the use of multiple drugs with different mechanisms of action may also be the basis for this synergism [6].

Opioid blockers did not alter the synergism that was induced by dexketoprofen and meloxicam because the I.I. was not significantly modified. The ab-

sence of an antinociceptive interaction between the opioid blockers and the NSAIDs may be due to the opioid concentrations used (mg/kg). It has been reported that opioid blockers induce antinociceptive activities by themselves only at ultra-low concentrations [5, 7, 23]. Studies have demonstrated that opioid blockers can enhance the activity of co-administered drugs, such as morphine, and, therefore, the doses normally needed to achieve antinociceptive activity can be reduced [1, 5].

Another reason why these blockers did not affect the activity of dexketoprofen and meloxicam may be because they did not alter the pharmacokinetic or metabolic parameters of these drugs. These results are in accordance with reports showing no effects of naltrexone on the synergism between combinations of NSAIDs with morphine [17]. Furthermore, similar ineffectiveness has been reported with naltrindole on a similar type of synergistic combination [16]. However, data are controversial with regards to norbinaltorphimine. It has been reported in some studies that this agent attenuates morphine antinociception [3, 8], but in other studies, no effect on morphine antinociception has been noted [24, 26]. In inflammatory orofacial pain, the antinociceptive interaction between meloxicam and ketoprofen and the role of K^+-Cl^- co-transporter 2 downregulation that is induced by formalin cannot be excluded [30]. However, new research exists on the role of PGs in peripheral and central sensitization processes that occur after injury. This research seems to open up new opportunities for using non-opioid analgesics for anti-inflammatory effects [21].

In conclusion, the present study shows that co-administering dexketoprofen and meloxicam produces synergic antinociception in both phases of the formalin orofacial assay. In addition, this antinociceptive activity does not seem to be modulated by the opioid system. This finding may be relevant in NSAID multi-modal analgesia where an opioid antagonist must be used.

Acknowledgment:
The expert technical assistance of José López and Alejandro Correa is greatly appreciated.

References:

1. Abul-Husn NS, Sutak M, Milne B, Jhamandas K: Augmentation of spinal morphine analgesia and inhibition of tolerance by low doses of μ- and -opioid receptor antagonists. Br J Pharmacol, 2007, 151, 877–887.
2. Barrera NP, Morales B, Torres S, Villalón M: Principles: Mechanisms and modeling of synergism in cellular responses. Trends Pharmacol Sci, 2005, 26, 526–532.
3. Broadbear JH, Negus SS, Butelman ER, De Costa BR, Woods JH: Differential effects of systemically administered nor-binaltorphimine (Nor-BNI) on kappa-opioid antagonists in the mouse writhing assay. Psychopharmacology (Berl), 1991, 115, 311–319.
4. Capone ML, Tacconelli S, Di Francesco L, Sacchetti A, Sciulli MG, Patrignani P: Pharmacodynamic of cyclooxygenase inhibitors in humans. Prostaglandins Other Lipid Mediat, 2007, 82, 85–94.
5. Choi SS, Han KJ, Lee HK, Han EJ, Suh HW: Possible antinociceptive mechanisms of opioid receptor antagonists in the mouse formalin test. Pharmacol Biochem Behav, 2003, 75, 447–457.
6. Chou TC: Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol Rev, 2006, 58, 621–681.
7. Crain SM, Shen KF: Acute thermal hyperalgesia elicited by low-dose morphine in normal mice is blocked by ultra-low dose naltrexone, unmasking potent opioid analgesia. Brain Res, 2001, 88, 75–82.
8. Endoh HT, Matsuura H, Tanaka C, Nagase H: Norbinaltorphimine: a potent and selective κ-opioid receptor antagonist with long-lasting activity in vivo. Arch Int Pharmacodyn Ther, 1992, 316, 30–42.
9. Goldstein A, Aronow L, Kalman SM: Principles of drug action. 2nd edn., J. Wiley & Sons, Inc., New York, 1974, 89–96.
10. Hilário MOE, Terreri MT, Len CA: Nonsteroidal anti-inflammatory drugs: cyclooxygenase 2 inhibitors. J Pediatr (Rio J), 2006, 82, 206–212.
11. Ito S, Okuda-Ashitaka E, Minami T: Central and peripheral roles of prostaglandins in pain and their interactions with novel neuropeptides nociceptin and nocistatin. Neurosci Res, 2001, 41, 299–332.
12. Jackson ID, Heidemann BH, Wilson J, Power L, Brown RD: Double-blind, randomized, placebo-controlled trial comparing rofecoxib with dexketoprofen trometamol in surgical dentistry. Br J Anaesth, 2004, 92, 675–680.
13. Luccarini P, Childeric A, Gaydier AM, Voisin D, Dallel R: The orofacial formalin test in the mouse: a behavioral model for studying physiology and modulation of trigeminal nociception. J Pain, 2006, 7, 908–914.
14. Malan TP Jr, Marsh G, Hakki SI, Grossman E, Traylor L, Hubbard RC: Parecoxib sodium, a parenteral cyclooxygenase 2 selective inhibitor, improves morphine analgesia and is opioid-sparing following total hip arthroplasty. Anesthesiology, 2003, 98, 950–956.
15. Mauleón D, Artigas R, García ML, Carganico G: 1996. Preclinical and clinical development of dexketoprofen. Drugs, 1996, 52, Suppl 5, 24–46.
16. Miranda HF, Pinardi G: Lack of effect of naltrindole on the spinal synergism between morphine and non-steroidal anti-inflammatory drugs (NSAIDs). J Physiol Pharmacol, 2009, 60, 71–76.

17. Miranda HF, Pinardi G: Lack of effect of naltrexone on the spinal synergism between morphine and non steroidal anti-inflammatory drugs. Pharmacol Rep, 2009, 61, 268–274.
18. Miranda HF, Puig MM, Dursteler C, Prieto JC, Pinardi G: Dexketoprofen-induced antinociception in animal models of acute pain: Synergy with morphine and paracetamol. Neuropharmacology, 2007, 52, 291–296
19. Miranda HF, Sierralta F, Prieto JC: Synergism between NSAIDs in the orofacial formalin test in mice. Pharmacol Biochem Behav, 2009, 92, 314–318
20. Satyanarayana PSV, Jain NK, Singh A, Kulkarni SK: Isobolographic analysis of interaction between cyclooxygenase inhibitors and tramadol in acetic acid-induced writhing in mice. Prog Neuropsychopharmacol Biol Psychiatry, 2004, 28, 641–649.
21. Schug SA, Monapas A: Update on the role of non-opioids for postoperative pain treatment. Best Pract Res Clin Anaesthesiol, 2007, 2, 15–30.
22. Shen KF, Crain SM: Ultra-low doses of naltrexone or etorphine increase morphine's antinociceptive potency and attenuate tolerance/dependence in mice. Brain Res, 1997, 757, 176–190.
23. Suleyman H, Demircan B, Karagoz Y: Anti-inflammatory and side effects of cyclooxygenase inhibitors. Pharmacol Rep, 2007, 59, 247–258.
24. Suzuki T, Narita M, Takahashi Y, Misawa M, Nagase H: Effects of nor-binaltorphimine on the development of analgesic tolerance to and physical dependence on morphine. Eur J Pharmacol, 1992, 213, 91–97.
25. Tallarida RJ: Drug synergism and dose-effect data analysis, Chapman & Hall CRC Press, Boca Raton, FL, 2000, 1–69.
26. Takemori AE, Ho BY, Naeseth JS, Portoghese PS: Nor-binaltorphimine, a highly selective kappa-opioid antagonist in analgesic and receptor binding assays. J Pharmacol Exp Ther, 1988, 246, 255–258.
27. Takemura M, Sugijo S, Moritami M, Kobayashi M, Yomehara N: Mechanisms of orofacial pain control in the central nervous system. Arch Histol Cytol, 2006, 69, 79–100.
28. Waldhoer M, Bartlett SE, Whistler JL: Opioid receptors. Annu Rev Biochem, 2004, 73, 953–990.
29. Wettstein JG, Grouhel A: Opioid antagonist profile of SC nor-binaltorphimine in the formalin paw assay. Pharmacol Biochem Behav, 1996, 53, 411–416.
30. Wu LA, Huang J, Wang W, Wang W, Wang XJ, Wu SX: Down-regulation of K^+-Cl^- co-transporter 2 in mouse medullary dorsal horn contributes to the formalin-induced inflammatory orofacial pain. Neurosci Lett, 2009, 457, 36–40.
31. Zegpi C, González C, Pinardi G, Miranda HF: The effect of opioid antagonists on synergism between dexketoprofen and tramadol. Pharmacol Res, 2009, 60, 291–295.

Received: February 16, 2010; **in the revised form:** October 19, 2010; **accepted:** November 18, 2010.