Available online at www.sciencedirect.com
ScienceDirect
Neuropharmacology 52 (2007) 291–296
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Dexketoprofen-induced antinociception in animal models of acute pain: Synergy with morphine and paracetamol

Hugo F. Miranda [a,*], Margarita M. Puig [b], Christian Dursteler [b], Juan Carlos Prieto [a,c], Gianni Pinardi [a,1]

[a] *Pharmacology Program, ICBM, Faculty of Medicine, University of Chile, Clasificador 70.000, Independencia 1027, Santiago 7, Chile*
[b] *Department of Anesthesiology, Hospital del Mar, Paseo Marítimo 25, 08003 Barcelona, Spain*
[c] *Cardiovascular Center, Hospital Clínico University of Chile, Chile*

Received 20 April 2006; received in revised form 29 June 2006; accepted 14 July 2006

Abstract

The antinociceptive activity of dexketoprofen was studied in mice using the acetic acid writhing test (acute tonic pain), the tail flick test (acute phasic pain) and the formalin assay (inflammatory pain). Isobolographic analysis was used to study the antinociceptive interactions between morphine and paracetamol co-administered with dexketoprofen. In the writhing test, the intraperitoneal administration of dexketoprofen or ketoprofen resulted in parallel dose–response curves with equal efficacy, but higher relative potency for dexketoprofen. In the tail flick test, the curves were parallel with similar efficacy and potency. The administration of morphine or paracetamol in both tests resulted in dose–response curves not parallel with that of dexketoprofen, which showed a potency between morphine and paracetamol. In the formalin assay, the antinociceptive activity of morphine during phase I was 122, 295 and 1695 times higher than dexketoprofen, ketoprofen and paracetamol, respectively. Isobolographic analysis demonstrated that the combination of sub-analgesic doses of dexketoprofen with morphine or with paracetamol was strongly synergic in all three tests. Synergistic drug combinations should improve effective pharmacological treatment of pain, minimizing drug specific adverse effects. These findings are undoubtedly worthy of additional controlled clinical trials in severe pain syndromes.

Keywords: Dexketoprofen; Ketoprofen; Morphine; Paracetamol; Antinociception; Writhing test; Tail flick test; Formalin assay; Isobologram; Synergy

1. Introduction

The administration of analgesics, either nonsteroidal anti-inflammatory drugs (NSAIDs) or opioids, induce antinociception in different animal pain models. It has been shown that the co-administration of racemic ketoprofen and morphine induced a synergistic analgesic interaction in the acetic acid writhing test of the mouse, which was not modified by opioid antagonists (Miranda et al., 2004). Racemic ketoprofen (KETO) is one of the most potent and selective inhibitors of cyclooxygenase-1 (COX-1) (Laudanno et al., 2002; Warner and Mitchell, 2004), and this effect is due to the *S*(+)-enantiomer, since the *R*(−)-enantiomer is devoid of such activity (Mauleon et al., 1996). The *S*(+)-dextrorotatory enantiomer of ketoprofen named dexketoprofen (DEX) has central analgesic actions in normal animals and depress nociceptive responses with a potency similar to that of μ-opioid agonists (Mazario et al., 1999). In addition, it is a very effective analgesic drug either in the normal situation or in monoarthritic rodents (Mazario et al., 2001).

The purpose of the present study is to evaluate the antinociceptive activity of dexketoprofen using two models of acute tonic pain, the acetic acid induced writhing test and the formalin test and one of phasic pain, the tail flick test (Le Bars et al., 2001). Also, this is the first investigation using isobolographic analysis to examine the combination of dexketoprofen with standard analgesics in writhing, tail flick and formalin paw assays.

* Corresponding author. Present address: Departamento de Matemática, Universidad del Bío-Bío, Casilla 1102, Concepción, Chile.
E-mail addresses: hmiranda@ubiobio.cl (H.F. Miranda), gpinardi@med.uchile.cl (G. Pinardi).
[1] Tel.: +56 2 678 6237; fax: +56 2 737 2783.

0028-3908/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2006.07.025

2. Material and methods

2.1. Animals

Male CF-1 mice (30 g), housed on a 12 h light–dark cycle at 22 ± 2 °C and with access to food and water ad libitum were used. Experiments were performed in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigation of experimental pain approved by the Animal Care and Use Committee of the Faculty of Medicine, University of Chile. Animals were acclimatized to the laboratory for at least 2 h before testing, were used only once during the protocol and were sacrificed immediately after the algesiometric test. The number of animals was kept at a minimum compatible with consistent effects of the drug treatments.

2.2. Writhing test

The procedure used has been described previously (Miranda et al., 2002). Mice were injected intraperitoneally (i.p.) with 10 mL/kg of 0.6% acetic acid solution, 30 min after the i.p. administration of the drugs, time at which preliminary experiments showed occurrence of the maximum effect. A writhe is characterized by a wave of contraction of the abdominal musculature followed by the extension of the hind limbs. The number of writhes in a 5 min period was counted, starting 5 min after the acetic acid administration. Antinociception was expressed as percent inhibition of the number of writhes observed in control animals (19.7 ± 0.30, $n = 25$).

2.3. Tail flick test

The algesiometric test was similar to that described previously (Pinardi et al., 2002, 2003). A radiant heat, automatic tail flick algesiometer (U. Basile, Comerio, Italy) was used to measure response latencies. The light beam was focused on the animal's tail about 4 cm from the tip and the intensity was adjusted so that baseline readings were between 2 and 3 s. An 8 s cut-off time was imposed to avoid damage to the tail. Control reaction time (latency of the response) was recorded twice, with an interval of 15 min between readings, the second reading being similar to the first. Only animals with baseline reaction times between 2 and 3 s were used in the experiments. Tail flick latencies were converted to % maximum possible effect (MPE) as follows:

$$\%\text{MPE} = \frac{(\text{postdrug latency} - \text{predrug latency})}{(\text{cut-off time} - \text{predrug latency})} 100$$

Each animal was used as its own control. Drugs were administered 30 min before the experimental protocol, a time at which preliminary experiments showed occurrence of the maximum effect. The dose that produced 25% of MPE (ED_{25}) was calculated from the linear regression analysis of the curve obtained by plotting log dose versus %MPE.

2.4. Formalin test

The method described by Rosland et al. (1990) was used. To perform the test, 20 μL of 5% formalin solution was injected into the dorsal surface of the mice right hind paw with a 27-gauge needle attached to a 50 μL Hamilton syringe. Each mouse was immediately returned to a plexiglass observation chamber. The degree of pain intensity was assessed as the total time spent by the animal licking or biting the injected paw, measured by visual observation and a digital time-out stopwatch. The test shows two clear cut phases; phase I corresponds to the 5 min period starting immediately after the formalin injection and represents a tonic acute pain due to peripheral nociceptor sensitization; in the present work, only the antinociception recorded during this phase was considered. Phase II was recorded as the 10 min period starting 20 min after the formalin injection and represents inflammatory pain. Drug or saline was administered to animals 30 min before formalin injection, time at which preliminary experiments showed occurrence of the maximum effect. Control animals ($n = 25$) were injected with saline. For each drug, analgesic effects were characterized after the administration of a minimum of four doses in logarithmic increments. The licking times observed were converted to % maximum possible effect (MPE) as follows:

$$\%\text{MPE} = 100 - \left[\frac{(100 \times \text{postdrug total licking time})}{\text{control total licking time}}\right]$$

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

2.5. Protocol

Dose–response curves for dexketoprofen (DEX), morphine (MOR) and paracetamol (PARA) were obtained using at least six animals at each of at least four doses. A least-squares linear regression analysis of the log dose–response curve allowed the calculation of the doses that produced 25 or 50% of antinociception when each drug was administered alone (ED_{25} for tail flick or ED_{50} for writhing and formalin tests). ED_{25} was used in the tail flick test as the equieffective dose instead of ED_{50} for isobolographic analysis because higher doses did not show increased effects without motor impairments (Pinardi et al., 2002). Then a dose–response curve was also obtained and analyzed after the co-administration of DEX with MOR or with PARA in combinations of fixed ratios based on the following fractions 1/2, 1/4, 1/8, 1/16 of their respective ED_{50} (writhing and formalin tests) or ED_{25} values (tail flick test).

An isobolographic analysis was used to characterize drug interactions. The method of isobolographic analysis has been described previously in detail (Miranda et al., 2002, 2004). The ED_{50} or ED_{25} for the drug combinations were obtained by linear regression analysis of the dose–response curves. In the case of the formalin assay, isobolographic analysis was performed only for phase I, because the responses on phase II were not clearly dose-dependent, and a proper log dose–response curve could not be analyzed. Supra-additivity or synergistic effect is defined as the effect of a drug combination that is higher and statistically different ($ED_{25\text{ or }50}$ significantly lower) than the theoretically calculated equieffect of a drug combination with the same proportions. If the $ED_{25\text{ or }50}$s 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 was calculated as the experimental $ED_{25\text{ or }50}$/the theoretical $ED_{25\text{ or }50}$. If the value is close to 1, the interaction is additive. Values lower than 1 are an indication of the magnitude of supra-additive or synergistic interactions and values higher than 1 correspond to sub-additive or antagonistic interactions (Tallarida, 2001).

2.6. Drugs

All drugs were freshly dissolved in saline. Dexketoprofen trometamol (DEX) was a gift from Menarini, Spain; ketoprofen (KETO) was provided by Rhone-Poulenc Rorer, Chile and paracetamol (PARA) by Bristol-Myers-Squibb, France; Morphine hydrochloride (MOR) was purchased from Sigma Chemical Co., St. Louis, MO, USA. Doses were expressed on the basis of the salts.

2.7. Statistical analysis

Results are presented as ED_{25} or ED_{50} values ± SEM or with 95% confidence limits (95% CL). The program used to perform statistical procedures was Pharm Tools Pro (version 1.27, The McCary Group Inc.). Statistical analysis of the parallelism of dose–response curves was performed according to Tallarida and Murray (1987). Results were analyzed by ANOVA followed by Student-Newman–Keuls test. P values less than 0.05 ($P < 0.05$) were considered significant.

3. Results

3.1. Tail flick and writhing tests

The i.p. administration of DEX and KETO in the writhing test and in the tail flick test induced statistically parallel

dose–response curves with similar efficacy, but with higher relative potency for DEX in the writhing test (2.27:1). In the tail flick assay, however, the dose–response curves were also statistically parallel with similar efficacy, but the relative potency was almost equal (Fig. 1A and B). Fig. 1C and D shows the dose–response curves for DEX, MOR and PARA in the writhing and tail flick tests, respectively. As can be seen, all the curves were statistically not parallel. Based on equieffective doses in the tail flick assay, MOR was approximately 35 and 76 times more potent than DEX and PARA, respectively and in the writhing test, MOR was approximately 122 and 411 times more potent than DEX and PARA. The ED_{25} or ED_{50} values and SEM for the antinociceptive effects of the drugs are shown in Table 1.

3.2. Formalin test

The systemic administration of four doses of DEX, KETO, MOR and PARA, 30 min prior to the hind paw formalin injection, induced dose-dependent antinociceptive activities during phase I of the test and ED_{50}s for MPE are shown in Table 1. In this assay, the antinociceptive activity of MOR was 122, 295 and 1695 times higher than DEX, KETO and PARA, respectively.

3.3. Interaction between dexketoprofen with morphine or with paracetamol

The interactions between the combinations of DEX with MOR and DEX with PARA at fixed ratios of ED_{25} fractions (in the tail flick test) or ED_{50} fractions (in writhing and formalin assays) were assessed by isobolographic analysis of the dose–response curves obtained after i.p. co-administration. The isobolograms indicate that synergistic interactions occurred between DEX and MOR and between DEX and PARA (ED_{50} experimental $< ED_{50}$ theoretical; $P < 0.05$) in all nociception tests, as can be seen in Figs. 2 and 3. Tables 2 and 3 show the experimental and the theoretical additive ED_{50} values for the combinations with their 95% CL and the combinations fixed ratios. In addition, the interaction index values were significantly different between all combinations for all tests ($P < 0.05$), with the exception of DEX/MOR in writhing and formalin assays. These results suggest the following rank of potencies for the combinations: DEX/PARA > DEX/MOR in the tail flick assay and DEX/MOR > DEX/PARA in the writhing and formalin tests (Tables 2 and 3).

4. Discussion

The results obtained in the present study confirm the dose-dependent antinociceptive activity of DEX, KETO, PARA and MOR in the acetic acid writhing test, tail flick test and phase I of the formalin assay previously described (Ossipov et al., 2000; Bonnefont et al., 2003; Miranda et al., 2004; Tham et al., 2005). The formalin test is an algesiometric model designed to evaluate analgesic and anti-inflammatory activities and shows a distinct biphasic response: a short-lasting pain caused by a direct sensibilization effect on nociceptors, referred as phase I, and a longer lasting pain due to the inflammation process called phase II (Rosland et al., 1990). It is important to note that DEX, KETO and PARA were less potent

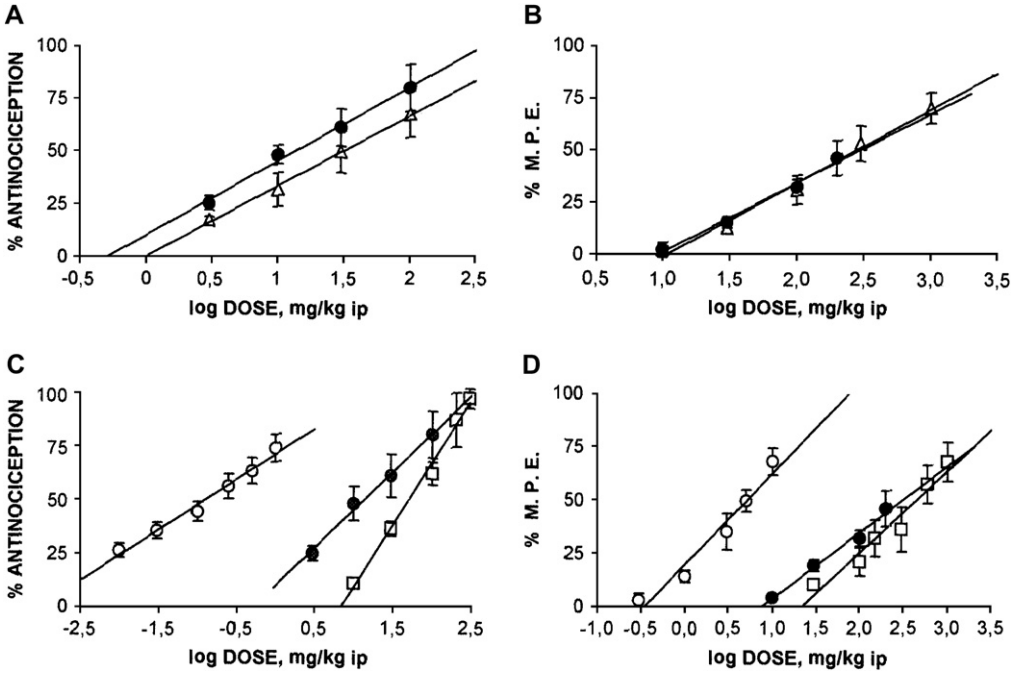

Fig. 1. Panel A: Dose–response curves for the antinociceptive effect of dexketoprofen (●) and ketoprofen (△) in the writhing test. Panel B: Dose–response curves for the antinociceptive effect of dexketoprofen (●) and ketoprofen (△) in the tail flick test. Panel C: Dose–response curves for the antinociceptive effect of dexketoprofen (●), morphine (○) and paracetamol (□) in the writhing test. Panel D: Dose–response curves for the antinociceptive effect of dexketoprofen (●), morphine (○) and paracetamol (□) in the tail flick test. % MPE = % maximum possible effect.

Table 1
ED_{25} and ED_{50} values ± SEM (mg/kg) for the antinociceptive effect of morphine and NSAIDs administered i.p. in the tail flick, writhing and formalin tests of mice

Drugs	Tail flick test ED_{25}	Writhing test ED_{50}	Formalin test ED_{50} phase I
Dexketoprofen	46.96 ± 4.32	14.67 ± 1.89	14.7 ± 3.1
Morphine	1.31 ± 0.54*	0.12 ± 0.012*	0.12 ± 0.05*
Ketoprofen	54.55 ± 6.01*	30.30 ± 3.85*	35.4 ± 7.3*
Paracetamol	99.84 ± 8.72*	49.40 ± 3.31*	203.4*

*Significantly different from dexketoprofen, $P < 0.05$.

in phase II than in phase I, which is in line with their poor anti-inflammatory profile as COX-1 as preferential inhibitors according to Warner and Mitchell (2004) (data not shown). The results obtained with DEX in the tail flick, writhing test and in phase I of the formalin assay, are in complete agreement with preclinical and clinical data obtained by Mauleon et al. (1996) and McGurk et al. (1998), who reported that a half a dose of dexketoprofen is at least as effective as twice a dose of racemic KETO. In the formalin assay, DEX was more potent than KETO to inhibit phase I. In contrast with the results of Ossipov et al. (2000), who reported that neither (*S*)-ketoprofen nor (*R*)-ketoprofen administered spinally significantly inhibited phase I of the formalin induced flinch response, in the present work the intraperitoneal administration of DEX and KETO inhibited the licking time response. Since the nociceptive response during phase I is thought to depend on peripheral sensitization of nociceptors (Rosland et al., 1990), spinally administered drugs may have no effect in this phase; however, in rats Yoon et al. (2006) have recently reported that intrathecally administered morphine and zaprinast showed synergy to inhibit phases I and II of the formalin test. On the other hand, it is known that ketoprofen in man may be subject to metabolic chiral inversion, principally from the *R*(−)-enantiomer to the more active *S*(+)-enantiomer (Rudy et al., 1998), the form responsible for the inhibition of cyclooxygenase (Carabaza et al., 1996); even if this bioinversion has been shown to be bidirectional in mice (Jamaly et al., 1997), pharmacokinetics factors may influence the results.

The fact that the increased synergy of DEX/MOR is higher in the writhing test than in tail flick and formalin, might indicate that, besides its central/spinal action, morphine in the writhing test may act also on an additional site on the gut to reduce the writhing response, since opioid receptors are present in the intestinal smooth muscle (Bodnar and Klein, 2005). The present findings, that DEX increased the analgesic effect of morphine are in agreement with those previously reported in an acute model of nociception, in which DEX enhanced the antinociception induced by another μ-agonist, fentanyl (Gaitan and Herrero, 2002, 2005). Additionally, a similar synergism was obtained with the combination of DEX with PARA, an expected result in concordance with the reported supra-additive activity displayed by the combination KETO/PARA in the writhing test (Miranda et al., 2006). The experimental ED_{50}s of the combination DEX/PARA in the present work are significantly lower than that reported by Miranda

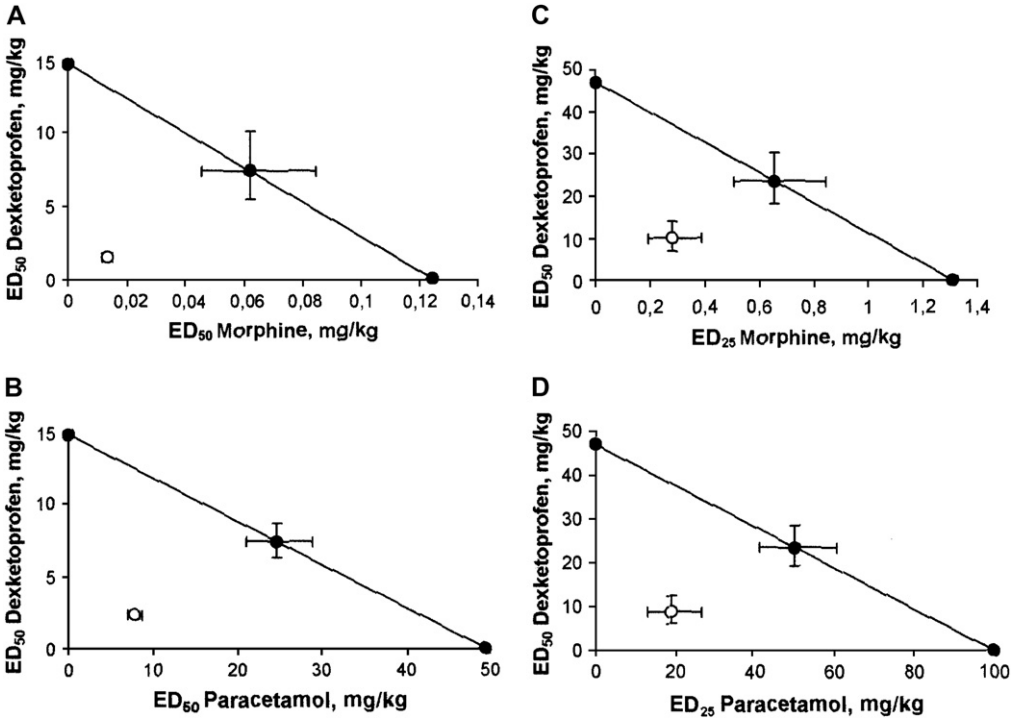

Fig. 2. Panels A and B: Isobolograms for the intraperitoneal administration of the combinations dexketoprofen/morphine (A) and dexketoprofen/paracetamol (B) in the writhing test. Panels C and D: Isobolograms for the intraperitoneal administration of the combinations dexketoprofen/morphine (C) and dexketoprofen/paracetamol (D) in the tail flick test. Filled circles (●) correspond to the theoretical ED_{50} or ED_{25} with 95% confidence limits and open circles (○) correspond to the experimental ED_{50} or ED_{25} with 95% confidence limits.

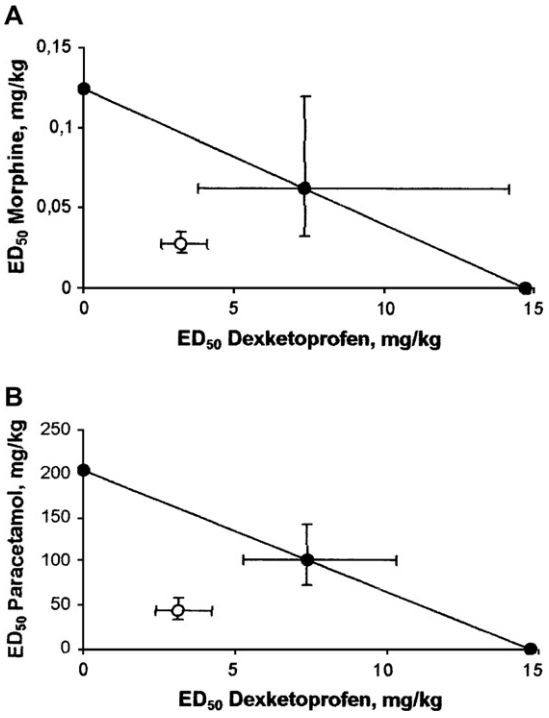

Fig. 3. Panels A and B: Isobolograms for the intraperitoneal administration of the combinations dexketoprofen/morphine and dexketoprofen/paracetamol in the formalin test. Filled circles (●) correspond to the theoretical ED_{50} with 95% confidence limits and open circles (○) correspond to the experimental ED_{50} with 95% confidence limits.

et al. (2006) for KETO/PARA. Furthermore, the comparison of the interaction indexes shows that the synergy is significantly higher when DEX instead of KETO is combined with PARA (Table 4).

The synergism observed with DEX/MOR and with DEX/PARA co-administrations, may be related with a central site of action, since it has been reported that DEX crosses the blood-barrier easily (Mazario et al., 1999), and it is well known that the analgesic effect of PARA is mainly central, inhibiting prostaglandin biosynthesis and activating serotonergic descending pathways (Graham and Scott, 2005; Pickering et al., 2006).

NSAIDs may exert their antinociceptive actions through other complex mechanisms, in addition to the inhibition of prostaglandin biosynthesis. These include modulations by endogenous opioids, serotonergic and noradrenergic mechanisms, as well as by the NO–GMPc pathway (Fürst, 1999; Pinardi et al., 2003; Miranda et al., 2001, 2002, 2003; Díaz-Reval et al., 2004). Different pathways activated by different NSAIDs to reduce nociceptive impulse transmission at different levels may lie at the basis of the synergic actions of combinations, since if only one pathway is activated, presumably the effect of co-administration would be only additive (Tallarida, 2001). An interaction at the level of signal transduction might be considered, since all prostanoids and opioid receptors are G-protein coupled receptors and if they coexist on neurons, they could share a common pool of G-protein. Thus, activation of one receptor may cause a redistribution

Table 2
Isobolographic parameters for the antinociceptive activity of the combinations dexketoprofen/morphine and dexketoprofen/paracetamol administered i.p. in the writhing and tail flick tests of mice

Combinations	DEX/MOR	DEX/PARA
Writhing test		
Theoretical ED_{50} mg/kg (CL)	7.40 (5.42–10.09)	32.03 (27.32–35.56)
Experimental ED_{50} mg/kg (CL)	1.58 (1.36–1.79)*	10.11 (9.01–11.28)*
Interaction index	0. 214	0.316
Drugs ratio	1: 0.008	1: 0.29
Tail flick test		
Theoretical ED_{25} mg/kg (95% CL)	24.13 (18.69–31.16)	73.40 (60.46–89.10)
Experimental ED_{25} mg/kg (95% CL)	10.25 (7.07–14.27)*	27.83 (19.22–39.08)*
Interaction index	0.425	0.379
Drugs ratio	1:0.027	1:0.470

DEX = dexketoprofen; MOR = morphine; PARA = paracetamol.
*$P < 0.05$ versus theoretical value.
Lower values of interaction index indicate higher potency of the drug combinations.

Table 3
Isobolographic parameters for the antinociceptive activity of the combinations dexketoprofen/morphine and dexketoprofen/paracetamol, administered i.p. in phase I of the formalin test

Combinations	DEX/MOR	DEX/PARA
Formalin test (phase I)		
Theoretical ED_{50} mg/kg (95% CL)	7.42 (3.83–14.37)	109.1(78.0–152.5)
Experimental ED_{50} mg/kg (95% CL)	3.62 (2.70–5.03)*	46.28 (25.95–75.97)*
Interaction index	0.487	0.424
Drugs ratio	1:0.008	1:0.072

DEX = dexketoprofen; MOR = morphine; PARA = paracetamol.
*$P < 0.05$ versus theoretical value.
Lower values of interaction index indicate higher potency of the drug combinations.

Table 4
Comparison between the antinociceptive activity of the combinations dexketoprofen/paracetamol and ketoprofen/paracetamol (Miranda et al., 2006) in the writhing and tail flick tests

Combinations	DEX/PARA	KETO/PARA
Writhing test		
Theoretical $ED_{50} \pm$ SEM, mg/kg	32.0 ± 1.9	35.8 ± 2.6
Experimental $ED_{50} \pm$ SEM, mg/kg	10.1 ± 0.3*	20.4 ± 1.1
Interaction index	0.316*	0.569
Drugs ratio	1:0.297	1:0.734
Tail flick test		
Theoretical $ED_{25} \pm$ SEM, mg/kg	73.4 ± 4.9	77.5 ± 4.4
Experimental $ED_{25} \pm$ SEM, mg/kg	27.8 ± 2.8*	47.8 ± 4.2
Interaction index	0.379*	0.618
Drugs ratio	1:0.470	1:0.553

DEX = dexketoprofen; PARA = paracetamol.
*$P < 0.05$ versus KETO/PARA.
Lower values of interaction index indicate higher potency of the drug combinations.

of its G-protein, which increases the sensitivity of the other receptor (Djellas et al., 2002). However, the exact mechanism of the synergistic interactions obtained in the present investigation cannot be established with the methodology of this study.

The findings that low doses of DEX may be used in combination with low doses of MOR or PARA to induce effective pain relief should represent a great clinical benefit. It has been suggested (Raffa et al., 2003) that synergistic drug combinations may improve the effective pharmacological treatment of pain, and the lower dose of each agent required will minimize drug specific adverse effects. A clinical study indicating that DEX markedly improved analgesia and decreased opioid requirements in hip arthroplasty has been published (Iohom et al., 2002). However, additional controlled clinical trials in severe pain syndromes are justified by these findings.

Acknowledgements

Partially supported by a grant from Fondo de Investigaciones Sanitarias # C03/06, Madrid, Spain. The expert technical assistance of J. López and A. Correa is gratefully acknowledged.

References

Bodnar, R.J., Klein, G.E., 2005. Endogenous opiates and behavior: 2004. Peptides 26, 2629–2711.

Bonnefont, J., Alloui, A., Chapuy, E., Clottes, E., Eschalier, A., 2003. Orally administered paracetamol does not act locally in the rat formalin test. Anesthesiology 99, 976–981.

Carabaza, A., Cabre, F., Rotllan, E., Gomez, M., Gutierrez, M., Garcia, M.L., Mauleon, D., 1996. Stereoselective inhibition of inducible cyclooxygenase by chiral nonsteroidal antiinflammatory drugs. J. Clin. Pharmacol. 36, 505–512.

Díaz-Reval, M.I., Ventura-Martinez, R., Deciga-Campos, M., Terron, J.A., Cabre, F., Lopez-Muñoz, F.J., 2004. Evidence for a central mechanism of action of *S*(+)-ketoprofen. Eur. J. Pharmacol. 483, 241–248.

Djellas, Y., Antonakis, K., Le Breton, G.C., 2002. Shifts in the affinity distribution of one class of seven-transmembrane receptors by activation of a separate class of seven-transmembrane receptors. Biochem. Pharmacol. 59, 1521–1529.

Fürst, S., 1999. Transmitters involved in antinociception in the spinal cord. Brain Res. Bull. 48, 129–141.

Gaitan, G., Herrero, J.F., 2002. Subanalgesic doses of dexketoprofen trometamol enhance the potency and duration of fentanyl antinociception. Br. J. Pharmacol. 135, 393–398.

Gaitan, G., Herrero, J.F., 2005. Subanalgesic doses of dexketoprofen and HCT-2037 (nitrodexketoprofen) enhance fentanyl antinociception in monoarthritic rats. Pharmacol. Biochem. Behav. 80, 327–332.

Graham, G.G., Scott, K.F., 2005. Mechanism of action of paracetamol. Am. J. Ther. 12, 46–55.

Iohom, G., Walsh, M., Higgins, G., Shorten, G., 2002. Effect of perioperative administration of dexketoprofen and opioid requirements and inflammatory response following elective hip arthroplasty. Br. J. Anaesth. 88, 520–526.

Jamaly, F., Lovlin, R., Aberg, G., 1997. Bi-directional chiral inversion of ketoprofen in CD-1 mice. Chirality 9, 29–31.

Laudanno, O.M., Piombo, G., Cesolaria, J.A., Godoy, A., Rocaspana, A., Aramberry, L., 2002. Dexketoprofene, selective COX-1 inhibitor NSAID, without gastrointestinal injury in rats. Acta Gastroenterol. Latinoam. 32, 17–20.

Le Bars, D., Gozariu, M., Cadden, S.W., 2001. Animals models of nociception. Pharmacol. Rev. 53, 597–652.

Mauleon, D., Artigas, R., Garcia, M.L., Carganico, G., 1996. Preclinical and clinical development of dexketoprofen. Drugs 52, 24–45.

Mazario, J., Roza, C., Herrero, J.F., 1999. The NSAID dexketoprofen trometamol is a potent as μ-opioids in the depression of wind-up and spinal cord nociceptive reflexes in normal rats. Brain Res. 816, 512–517.

Mazario, J., Gaitan, G., Herrero, J.F., 2001. Cyclooxygenase-1 versus cyclooxygenase-2 inhibitors in the induction of antinociception in rodent withdrawal reflexes. Neuropharmacology 40, 937–945.

McGurk, M., Robinson, P., Rajayogeswaran, V., De Luca, M., Casini, A., Artigas, R., Muñoz, G., Mauleon, D., 1998. Clinical comparison of dexketoprofen trometamol, ketoprofen, and placebo in postoperative dental pain. J. Clin. Pharmacol. 38, 46–54.

Miranda, H.F., Sierralta, F., Pinardi, G., 2001. An isobolographic analysis of the adrenergic modulation of diclofenac antinociception. Anesth. Analg. 93, 430–435.

Miranda, H.F., Sierralta, F., Pinardi, G., 2002. Neostigmine interactions with non steroidal anti-inflammatory drugs. Br. J. Pharmacol. 135, 1591–1597.

Miranda, H.F., Lemus, I., Pinardi, G., 2003. Effect of the inhibition of serotonin biosynthesis on the antinociception induced by nonsteroidal anti-inflammatory drugs. Brain Res. Bull. 61, 417–425.

Miranda, H.F., Silva, E., Pinardi, G., 2004. Synergy between the antinociceptive effects of morphine and NSAIDs. Can. J. Physiol. Pharmacol. 82, 331–338.

Miranda, H.F., Puig, M.M., Prieto, J.C., Pinardi, G., 2006. Synergism between paracetamol and nonsteroidal anti-inflammatory drugs in experimental acute pain. Pain 121, 22–28.

Ossipov, M.H., Jerussi, T.P., Ren, K., Sun, H., Porreca, F., 2000. Differential effects of spinal (*R*)-ketoprofen and (*S*)-ketoprofen against signs of neuropathic pain and tonic nociception: evidence for a novel mechanism of action of (*R*)-ketoprofen against tactile allodynia. Pain 87, 193–199.

Pickering, G., Loriot, M.A., Libert, F., Eschalier, A., Beaune, P., Dubray, C., 2006. Analgesic effect of acetaminophen in humans: first evidence of a central serotonergic mechanism. Clin. Pharmacol. Ther. 79, 371–378.

Pinardi, G., Sierralta, F., Miranda, H.F., 2002. Adrenergic mechanism in antinociceptive effects of non steroidal anti-inflammatory drugs in acute thermal nociception in mice. Inflamm. Res. 51, 219–222.

Pinardi, G., Sierralta, F., Miranda, H.F., 2003. Atropine reverses the antinociception of nonsteroidal anti-inflammatory drugs in the tail-flick test of the mice. Pharmacol. Biochem. Behav. 74, 603–608.

Raffa, R.B., Clark-Vetri, R., Tallarida, R.J., Wertheimer, A.I., 2003. Combination strategies for pain management. Expert Opin. Pharmacother. 4, 1697–1708.

Rosland, J.H., Tjolsen, A., Maehle, B., Hole, K., 1990. The formalin test in mice, effect of formalin concentration. Pain 42, 235–242.

Rudy, A.C., Liu, Y., Brater, C., Hall, S.D., 1998. Stereoselectivity pharmacokinetics and inversion of (*R*)-ketoprofen in healthy volunteers. J. Clin. Pharmacol. 38 (Suppl. 2), 3S–10S.

Tallarida, R.J., Murray, R.B., 1987. Manual of Pharmacological Calculations. Springer-Verlag, New York, p. 19.

Tallarida, R.J., 2001. Drug synergism: its detection and applications. J. Pharmacol. Exp. Ther. 298, 865–872.

Tham, S.M., Angus, J.A., Tudor, E.M., Wright, C.E., 2005. Synergistic and additive interactions of the cannabinoid agonist CP55,940 with μ opioid receptors and α_2-adrenoceptor agonists in acute pain models in mice. Br. J. Pharmacol. 144, 875–884.

Warner, T.D., Mitchell, J.A., 2004. Cyclooxygenases, new forms, new inhibitors, and lessons from the clinic. FASEB J. 18, 790–804.

Yoon, M.H., Choy, J.I., Kim, S.J., Kim, C.M., Bae, H.B., Chung, S.T., 2006. Synergistic antinociception between zaprinast and morphine in the spinal cord of rats on the formalin test. Eur. J. Anaesthesiol. 23, 65–70.