Pharmacology, Biochemistry and Behavior 97 (2011) 423–427

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

# Interaction between dexibuprofen and dexketoprofen in the orofacial formalin test in mice

H.F. Miranda [a,b,*], V. Noriega [b], F. Sierralta [a], J.C. Prieto [a,c]

[a] School of Medicine, Pharmacology Program, ICBM, Faculty of Medicine, Universidad de Chile, Clasificador 70.000, Santiago 7, Chile
[b] Faculty of Medicine, School of Pharmacy, Universidad Nacional Andrés Bello, Santiago, Chile
[c] Cardiovascular Department, Hospital Clínico, Universidad de Chile, Santiago, Chile

ARTICLE INFO

*Article history:*
Received 2 August 2010
Received in revised form 13 September 2010
Accepted 16 September 2010
Available online 29 Septeber 2010

*Keywords:*
Orofacial pain
NSAIDs
Dexketoprofen
Dexibuprofen
Synergism

ABSTRACT

Animal models are used to research the mechanisms of pain and to mimic human pain. The purpose of this study was to determine the degree of interaction between dexketoprofen and dexibuprofen, by isobolographic analysis using the formalin orofacial assay in mice. This assay presents two-phase time course: an early short-lasting, phase I, starting immediately after the formalin injection producing a tonic acute pain, leaving a 15 min quiescent period, followed by a prolonged, phase II, after the formalin and representing inflammatory pain. Administration of dexketoprofen or dexibuprofen produced a dose-dependent antinociception, with different potency, either during phases I or II. The co-administration of dexketoprofen and dexibuprofen produced synergism in phase I and II. In conclusion, both dexketoprofen and dexibuprofen are able to induce antinociception in the orofacial formalin assay. Their co-administration produced a synergism, which could be related to the different degree of COX inhibition and other mechanisms of analgesics.



## 1. Introduction

Animal models of tissue injury have been used to research the mechanisms of pain and to mimic human pain. Preclinical studies using different algesiometric models have provided insights into the mechanisms, as well as the pharmacological treatment to control pain (Kim et al., 2010). Despite the great variety of analgesics available for the treatment of pain in humans, they seem to be inadequate. Most of the non-steroidal anti-inflammatory drugs (NSAIDs) which are effective in several models of pain, have a chiral centre. Thus, the enantiomer with S(+) configuration almost exclusively possesses the ability to inhibit prostaglandin activity. In contrast, R(−) enantiomers of NSAIDs have poor COX inhibitory activity. However, some R(−) enantiomers are not inert, and have different actions. Several preclinical and clinical studies have shown that chirally pure NSAIDs like dexketoprofen, dexibuprofen and S(+) etodolac are more potent than their respective R(−) enantiomers. Favourable pharmacokinetic and pharmacodynamic profiles of dexketoprofen, dexibuprofen and S(+) etodolac make them effective and well tolerated drugs for the treatment of painful inflammatory conditions, at half doses of racemate. Thus, chiral switch of NSAIDs is a rational approach for the treatment of painful inflammatory conditions (Hardikar, 2008).

Dexketoprofen trometamol is a water-soluble salt of the dextrorotatory enantiomer of the NSAID ketoprofen. Racemic ketoprofen is used as an analgesic and anti-inflammatory agent, and is one of the most potent in vitro inhibitors of prostaglandin synthesis. This effect is due to the S(+) enantiomer (dexketoprofen), while the R(−) enantiomer is devoid of such activity (Mauleón et al., 1996). On the other hand, dexibuprofen may be classified as an effective and highly tolerable drug against inflammation and pain (Zohmann et al., 1998). Furthermore, dexibuprofen is at the same level as modern NSAIDs, combining the high efficacy of diclofenac with the good tolerability of ibuprofen, and need not hide behind the new generation of COX-2 inhibitors (Phleps, 2001). The S(+) ibuprofen was found to be more potent than the racemic formulation and produced less acute gastric damage (Bonabello et al., 2003).

The purpose of this study was to determine the degree of interaction (i.e. synergistic or additive) between dexketoprofen and dexibuprofen. The type of interaction was evaluated by means of the isobolographic analysis using the formalin orofacial assay in mice. This test was selected since the face and mouth have a special biological, emotional and psychological value for every individual. Additionally, the face and mouth represent places in the body where most of the pain occurs.

## 2. Materials and methods

In all experiments CF-1 male mice of 35–40 days of age, weighing 29 ± 1.0 g, housed in a 12 h light–dark cycle at 22 ± 1 °C, with free

* Corresponding author. Pharmacology Program, ICBM, Faculty of Medicine, Universidad de Chile, Clasificador 70.000, Santiago 7, Chile. Tel.: +56 2 978 6237; fax: +56 2 737 2783.
*E-mail address:* hmiranda@med.uchile.cl (H.F. Miranda).

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.09.017

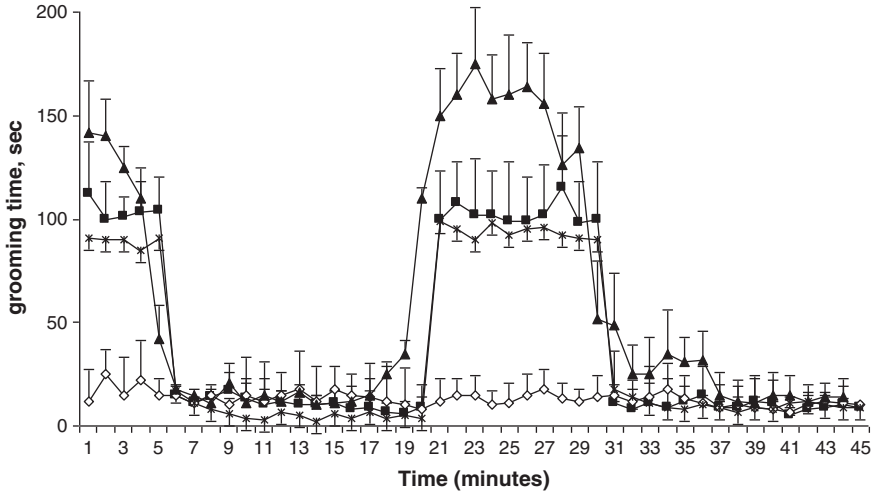


**Fig. 1.** Time course for the grooming activity of the orofacial formalin test in mice. Saline (◇), (✳) formalin 1%, (■) formalin 2%, (▲) formalin 5%. Each point represents the mean with S.E.M. of at least 8 mice.

access to food and water were used. The animals were acclimatized to the laboratory environment for at least 2 h before use. Experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and experimental procedures were approved by the Institutional Animal Care and Use Committee at the Universidad de Chile, Santiago, Chile. Each animal was used only once and received only one dose of the drugs tested. All drugs were freshly prepared by dissolving in normal saline and administered intraperitoneally (i.p.). All observations during the assay were performed by the authors in a randomized and blind manner. Control saline animals were run interspersed concurrently with the drug-treated animals (at least two mice per group), which prevented all the controls being run on a single group of mice at one time during the course of the research.

### 2.1. Orofacial formalin test

The method described by Luccarini et al. (2006) was used. To perform the test, 20 μl of 5% formalin solution were injected into the upper right lip of each mouse, with a 27 ga needle. In preliminary experiments, different groups of mice were treated with diverse concentrations of formalin (1, 2 or 5%) to establish the concentration-response ratio for both phases. Based on these results, we selected the formalin 5%, since with this concentration it was easy to detect inhibitory treatment. After the injection of formalin, the mice were immediately returned to a glass observation chamber. The intensity of pain was determined by the total time that the animal spent rubbing its lip with one of its extremities. Analgesics (NSAIDs) or saline solution in the control group, were administered 30 min before the formalin injection. Two phases can be distinguished during the assay; phase I corresponds to the 5 min period starting immediately after formalin injection and represents a 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. Each drug effects were characterized after the administration of at least 4 doses in logarithmic increments. The nociceptive score was determined for each phase by converting the total seconds the animal spent grooming into a percentage of maximum possible effect (MPE), as follows:

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

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

### 2.2. Protocol

Dose–response curves for i.p. administration of dexketoprofen or dexibuprofen were obtained using at least six animals for each with at least four doses. Linear regression analysis of the log dose–response curve allowed the calculation of the doses that produced 50% of antinociception ($ED_{50}$), when each drug was administered alone. $ED_{50}$ was used in the orofacial formalin tests as the equieffective dose for isobolographic analysis, because higher doses did not show increased effects without motor impairment (Miranda et al., 2009). Then a

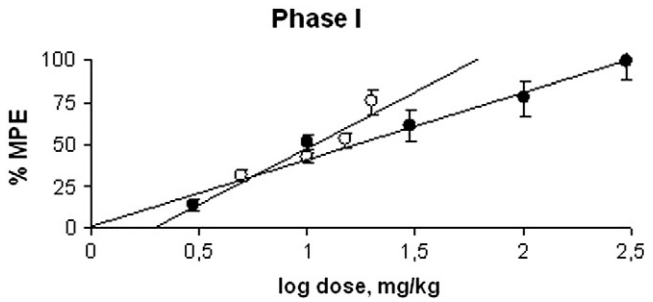


**Fig. 2.** Dose-effect curves for the antinociceptive activity induced by intraperitoneal administration of dexketoprofen (●) and dexibuprofen (○), in phase I of the formalin orofacial assay in mice. Each point represents the mean ± S.E.M of 6–8 mice. MPE: % maximum possible effect.

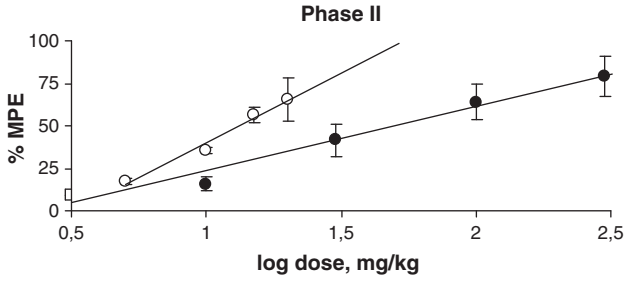


**Fig. 3.** Dose-effect curves for the antinociceptive activity induced by intraperitoneal administration of dexketoprofen (●) and dexibuprofen (○), in the phase II of the formalin orofacial assay of the mice. Each point represents the mean ± S.E.M of 6–8 mice. MPE: % maximum possible effect.

**Table 1**
$ED_{50}$ values with S.E.M. for the antinociceptive activity of intraperitoneal administration of dexketoprofen and dexibuprofen in phase I and phase II of the orofacial formalin test in mice.

| Drugs | $ED_{50} \pm$ S.E.M. (mg/kg i.p.) | |
|---|---|---|
| | Phase I | Phase II |
| Dexketoprofen | $17.51 \pm 3.29$ | $52.74 \pm 7.87$[a] |
| Dexibuprofen | $11.01 \pm 1.24$ | $13.26 \pm 0.71$[b] |

[a] $P<0.05$ compared with phase I.
[b] $P<0.05$ compared with phase II.

similar dose–response curve was also obtained and analyzed after the co-administration of dexketoprofen and dexibuprofen, in fixed ratio (1:1) combinations based on the mixture of 1/2, 1/4, 1/8, 1/16 of their respective $ED_{50}$ values.

### 2.3. Isobolographic analysis

Isobolographic analysis was used to characterize drug interactions. The method of isobolographic analysis has been described previously in detail (Miranda et al., 2009). The isobologram was constructed by connecting the $ED_{50}$ of the dexketoprofen plotted on the abscissa with the $ED_{50}$ of dexibuprofen plotted on the ordinate to obtain the additive line. For the drug mixture, the $ED_{50}$ and its associated 95% confidence intervals (CL) were determined by linear regression analysis of the log dose–response curve (eight animals at each with at least four doses) and compared by a 't'-test to a theoretical additive $ED_{50}$ obtained from calculating:

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

where R is the potency ratio of the NSAID alone compared to dexketoprofen alone, P1 is the proportion of dexibuprofen and P2 is the proportion of dexketoprofen in the total mixture. In the present study, fixed-ratio proportions were selected, first by combining the $ED_{50}$ of each compound and then constructing a dose–response curve in which $ED_{50}$ fractions (1/2, 1/4, 1/8 and 1/16) of the dexketoprofen and dexibuprofen combination were administered; in the equation above, $ED_{50}$ add is the total dose and the variance of $ED_{50}$ add was calculated from the fraction of the $ED_{50}$'s (i.e. 0.5) in the combination as:

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

From these variances, confidence limits are calculated and resolved according to the ratio of the individual drugs in the combination. The $ED_{50}$ for the drug combinations was obtained by linear regression analysis of the dose–response curves. Supraadditivity 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 equieffect of a drug combination with the same proportions. If the $ED_{50}$'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 (I.I.) was calculated as the experimental $ED_{50}$/the theoretical $ED_{50}$. 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 (Miranda et al., 2007, 2009).

### 2.4. Drugs

All drugs were freshly dissolved in saline on a constant volume of 10 ml/kg and administered intraperitoneally (i.p.) at doses of 3–300 mg/Kg for dexketoprofen or 3–30 mg/Kg for dexibuprofen. Dexketoprofen was donated by Menarini Laboratories, Spain, and dexibuprofen was donated by Labomed Farmaceútica, Chile.

### 2.5. Statistical analysis

Results are presented as mean ± S.E.M. or as $ED_{50}$ values with 95% confidence limits (95% CL). Isobolographic calculations were performed with the program Pharm Tools Pro (version 1.27, The McCary Group Inc. PA, USA), based on Tallarida (2000). Statistical analysis of the isobolograms was performed according to Tallarida (2000) and the difference between experimental and theoretical values was assessed by Student's *t* test for independent means. *P* values under 0.05 ($P<0.05$) were considered significant.

## 3. Results

### 3.1. Nociceptive behavioural response

The time course of the nociceptive response to the orofacial formalin test is shown on Fig. 1. Orofacial formalin 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. Phase II was recorded as the 10-min period starting 20 min after the formalin injection and represents inflammatory pain. The different doses of NSAIDs used did not induce significant motor dysfunction in the animals tested.

### 3.2. Antinociception produced by NSAIDs

The i.p. administration of dexketoprofen or dexibuprofen produced a dose-dependent antinociceptive activity with different potency in both, phases I and II of the formalin orofacial assay (see Figs. 2 and 3). The corresponding $ED_{50}$ values of dexketoprofen and dexibuprofen are summarized in Table 1.

### 3.3. Interaction between dexketoprofen and dexibuprofen

The interaction between dexketoprofen and dexibuprofen on the basis of the fixed ratio (1:1) of their $ED_{50}$ values alone was evaluated by isobolographic analysis. The theoretical additive $ED_{50}$ and the experimental $ED_{50}$ values for the fixed ratio combination are shown in Table 2.

Co-administration of dexketoprofen with dexibuprofen produced supra-additive or synergistic interaction in phase I and phase II, see

**Table 2**
$ED_{50}$ values (theoretical and experimental) in mg/kg, with 95% confidence limits (CL) for the antinociceptive activity of intraperitoneal administration of dexketoprofen with dexibuprofen in phase I and phase II of the orofacial formalin test in mice.

| Drug | $ED_{50}$ phase I | | $ED_{50}$ phase II | |
|---|---|---|---|---|
| | Theoretical | Experimental | Theoretical | Experimental |
| Dexketoprofen plus dexibuprofen | 14.26 (9.8–20.6)[a] | 8.01 (13.4–5.3)[a] | 33.00 (47.1–23.1) | 18.70 (29.9–13.8) |

[a] $P<0.05$ compared with phase II.

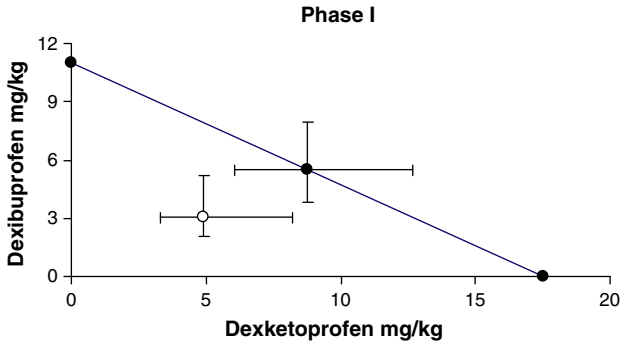


**Fig. 4.** Isobologram of the antinociception induced by intraperitoneal administration of the combination of dexketoprofen and dexibuprofen in phase I of the formalin orofacial assay in mice. (●) the theoretical $ED_{50}$ with 95% CL and (○) experimental $ED_{50}$ with 95% CL.

Figs. 4 and 5. In addition, the interaction indexes obtained are similar in both phases: 0.562 for phase I and 0.567 for phase II.

## 4. Discussion

This work confirms that administration of formalin in mice, as well as in rats, induces a behavioural response consisting of a typical biphasic response, as seen in all formalin assays (Raboisson and Dallel, 2004; Miranda et al., 2009). Phase I results from a direct stimulation of nociceptors, whereas phase II involves a period of sensitization during which inflammatory phenomena occur through peripheral mechanisms (Le Bars et al., 2001). In agreement with Luccarini et al. (2006) a dose-dependent nociceptive effect, in phase I and in phase II, was observed with the administration of formalin from 1% to 5%. In these experiments, we used 5% formalin, because with this concentration, the behavioural response to NSAIDs was easy to detect.

The intraperitoneal administration of dexketoprofen, dexibuprofen and their combination produced a dose-dependent effect in both phases of the formalin assay. In addition, higher potency of both NSAIDs in phase I was detected however higher potency of dexibuprofen when compared to dexketoprofen in both phases I and II was detected. Theoretically, dexketoprofen is expected to provide equivalent analgesia to ketoprofen at half the dose, since dexketoprofen is the (S)-enantiomer, which is believed to produce analgesia, with a consequent reduction in gastrointestinal adverse events (Barden et al., 2009). Furthermore, dexibuprofen (S-isomer ibuprofen), compared with ibuprofen, reduces gastric damage and improves analgesic and anti-inflammatory effects in rodents (Bonabello et al., 2003). In the present study, this assumption is reinforced for both phases since the experimental $ED_{50}$ of the mixture dexketoprofen with dexibuprofen was 8.01 mg/Kg in phase I and 18.72 mg/Kg in phase II, whereas the experimental $ED_{50}$ of the racemic mixture (ketoprofen with ibuprofen) was 14.58 m/kg in phase I and 18.85 mg/Kg in phase II (data not shown). Consequently, the combination dexketoprofen with dexibuprofen, tested in the orofacial formalin assay produced a synergistic interaction in both phase I and phase II. The similar interaction indexes obtained, as expression of the magnitude of the interaction, may be related to the COX selectivity of each NSAIDs. Dexketoprofen has been classified as relatively COX-1 selective, whereas dexibuprofen is a COX-1 and COX-2 inhibitor (Boneberg et al., 1996; Jackson et al., 2004; Curry et al., 2005).

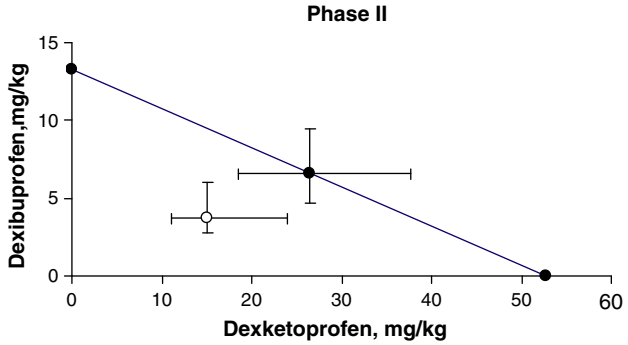


**Fig. 5.** Isobologram of the antinociception induced by intraperitoneal administration of the combination of dexketoprofen and dexibuprofen in phase II of the formalin orofacial assay in mice. (●) the theoretical $ED_{50}$ with 95% CL and (○) experimental $ED_{50}$ with 95% CL.

On the other hand, the findings of the present work, are not in agreement with those reported by Raboisson and Dallel (2004), because not only high doses of NSAIDs induced significant antinociception in both phases. Nevertheless, the synergism displayed by the combination of dexketoprofen with dexibuprofen, is consistent with other similar interactions in the same assay i.e., synergism between NSAIDs (Miranda et al., 2009); synergism between COX-3 inhibitors (Muñoz et al., 2010) or the synergistic antinociceptive effect of the co-administration of meloxicam and aminoguanidine in formalin-induced paw licking model in mice (Dudhgaonkar et al., 2008).

Despite the occurrence of synergism between analgesic drugs, the molecular mechanisms are not clear. It has been proposed that virtually all levels of cell function are involved (Barrera et al., 2005). Furthermore, it has been postulated that the use of multiple drugs with different mechanism of action may be the basis of synergism (Chou, 2006).

On the other hand, NSAIDs play an increasing role in the treatment of pain conditions, either individually for mild or moderate pain or in combination with other analgesic, as a component of multimodal analgesia.

In conclusion, both dexketoprofen and dexibuprofen are able to induce antinociception in the orofacial formalin assay. In addition, their co-administration produced a synergistic antinociceptive effect. This action could be related to the inhibition of COXs and other mechanisms of analgesics.

The findings of the present work may be immediate clinical application or relevance, for instance, in terms of new drug formulations.

## Acknowledgment

The expert technical assistance of José López and Alejandro Correa is gratefully acknowledged.

## References

Barden J, Derry S, McQuay HJ, Moore RA. Single dose oral ketoprofen and dexketoprofen for acute postoperative pain in adults. Cochrane Database Syst Rev 2009;7(4): CD007355 Oct.

Barrera NP, Morales B, Torres S, Villalón M. Principles: mechanisms and modeling of sinergism in cellular response. Trends Pharmacol Sci 2005;26:232–56.

Bonabello A, Galmozzi MR, Canaparo R, Isaia GC, Serpe L, Muntoni E, et al. Dexibuprofen (S+-isomer ibuprofen) reduces gastric damage and improves analgesic and anti-inflammatory effects in rodents. Anesth Analg 2003;97:402–8.

Boneberg EM, Zou MH, Ullrich V. Inhibition of cycloxigenase-1 and −2 by R(−)−and S(+) ibuprofen. J Clin Pharmacol 1996;36(12 Suppl):16S–9S.

Chou TC. Theoretical basis, experimental design, and computerized simulation of sinergysm and antagonism in drug combination studies. Pharmacol Rev 2006;58: 621–81.

Curry SL, Cogar SM, Cook JL. Nonsteroidal antinflammatory drugs: a review. J Am Anim Hosp Assoc 2005;41:298–309.

Dudhgaonkar SP, Tandan SK, Kumar D, Arunadevi R, Prakash VR. Synergistic interaction between meloxicam and aminoguanidine in formalin-induced nociception in mice. Eur J Pain 2008;12:321–8.

Hardikar MS. Chiral non-steroidal anti-inflammatory drugs a review. J Indian Med Assoc 2008;106:615–8.

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;92:675–80.
Kim H, Sung B, Mao J. Animal models of acute surgical pain. Meth Mol Biol 2010;617:31–9.
Le Bars D, Gozariu M, Cadden S. Animals model of nociception. Pharmacol Rev 2001;53: 597–652.
Luccarini P, Childeric A, Gayder 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;7:908–14.
Mauleón D, Artigas R, García ML, Carganico G. Preclinical and clinical development of dexketoprofen. Drugs 1996(Suppl. 5):24–46.
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;52:291–6.
Miranda HF, Sierralta F, Prieto JC. Synergism between NSAIDs in the orofacial formalin test in mice. Pharmacol Biochem Behav 2009;92:314–8.
Muñoz J, Navarro C, Noriega V, Pinardi G, Sierralta F, Prieto JC, et al. Synergism between COX-3 inhibitors in two animal models of pain. Inflammopharmacology 2010;18: 65–71.
Phleps W. Overview on clinical data of dexibuprofen. Clin Rheumatol 2001;20(Suppl 1):S15–21.
Raboisson P, Dallel R. The orofacial formalin test. Neurosci Biobehav Rev 2004;28: 219–26.
Tallarida RJ. Drug synergism and dose-effect data analysis. Boca Ratón, FL: Chapman & Hall/CRC Press; 2000. p. 26-131.
Zohmann A, Hawel R, Klein G, Kullich W, Lötsch G. S(+)−ibuprofen (dexibuprofen) excellent tolerance has not to be combined with poor clinical efficacy. Inflammopharmacology 1998;6:75–9.