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Synergism between paracetamol and nonsteroidal anti-inflammatory drugs in experimental acute pain

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Abstract

The antinociception induced by the intraperitoneal coadministration of combinations of paracetamol with the nonsteroidal anti-inflammatory drugs (NSAIDs) diclofenac, ibuprofen, ketoprofen, meloxicam, metamizol, naproxen, nimesulide, parecoxib and piroxicam was studied by isobolographic analysis in the acetic acid abdominal constriction test of mice (writhing test). The effective dose that produced 50% antinociception (ED_{50}) was calculated from the log dose–response curves of fixed ratio combinations of paracetamol with each NSAID. By isobolographic analysis, this ED_{50} was compared to the theoretical additive ED_{50} calculated from the ED_{50} of paracetamol and of each NSAID alone obtained from ED_{50} dose–response curves. As shown by isobolographic analysis, all the combinations were synergistic, the experimental ED_{50}s being significantly smaller than the theoretically calculated ED_{50}s. The results of this study demonstrate potent interactions between paracetamol and NSAIDs and validate the clinical use of combinations of these drugs in the treatment of pain conditions.

Keywords: Antinociception; NSAIDs; Paracetamol; Synergy; Abdominal constriction test; Writhing test; Isobologram

1. Introduction

The combination of analgesics of proven efficacy is a strategy intended to achieve one or more therapeutic goals, such as facilitating patient compliance, simplifying prescribing, improving efficacy without increasing adverse effects or decreasing adverse effects without loss of efficacy (Raffa, 2001; Hyllested et al., 2002). In certain cases, the coadministration of antinociceptive agents results in synergistic effects and the doses of the individual drugs can be substantially reduced (Maves et al., 1994; Salazar et al., 1995; Fairbanks and Wilcox, 1999; Kolesnikov et al., 2002; Miranda and Pinardi, 2004).

The antinociceptive action of nonsteroidal anti-inflammatory drugs (NSAIDs) is primarily due to the inhibition of prostaglandin biosynthesis through the inhibition of cyclooxygenase enzymes: COX-1 (constitutive) and COX-2 (inducible in inflammatory processes), even if alternative mechanisms have to be considered (Mitchell and Warner, 1999; Smith et al., 2000; Miranda et al., 2002; Warner and Mitchell, 2004). However, the absolute separation between the physiological and pathological roles of COX-1 and COX-2 is becoming less tenable and indeed their activities overlap to a considerable degree (Wallace, 1999). On the other hand, the mechanism of action of one of the most widely used analgesics, paracetamol or acetaminophen, remains largely unknown and at most the drug is considered to be an atypical NSAID, since it is a weak inhibitor of COXs (Botting, 2003). The following mechanisms have been postulated to explain paracetamol-induced

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analgesia: selective inhibition of cyclooxygenase activity in the CNS, interaction with spinal 5-HT_3 receptors, interference with spinal substance P receptors or inhibition of neurons excited by substance P, activation of suprasegmental descending inhibitory pathways, increase in pituitary β-endorphin secretion, direct effects on neuronal membrane potentials (Bannwarth et al., 1995; Pelissier et al., 1995; Pini et al., 1996; Raffa and Codd, 1996; Breivik et al., 1999).

NSAIDs and paracetamol are drugs widely used to treat moderate to mild pain, but they are often inadequate against severe pain. Clinical studies on patients with musculoskeletal conditions, dental pain or postoperative pain have shown that combinations of paracetamol and NSAIDs may provide additive pain-relief (Altman, 2004). However, a detailed evaluation of different combinations of paracetamol with commonly used NSAIDs has not been reported. The purpose of the present study was to assess the nature of the interaction between the systemic administration of combinations of paracetamol and several NSAIDs in a rodent model of visceral pain. A broad number of clinical relevant NSAIDs are included because they are the most frequently used agents for the treatment of pain (Møiniche et al., 2003; Rømsing and Møiniche, 2004).

2. Materials and methods

2.1. Animals

Male CF-1 mice (28–30 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 killed by cervical dislocation immediately after the algesiometric test. The number of animals was kept at a minimum compatible with consistent effects of the drug treatments.

2.2. Measurement of analgesic activity

Analgesic activity was assessed by the acetic acid abdominal constriction test (writhing test), a chemical visceral pain model (Hayashi and Takemori, 1971). Mice were injected i.p. with 10 mL/kg of 0.6% acetic acid solution 30 min after the i.p. administration of the drugs, a 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 acetic acid administration. Antinociceptive activity was expressed as percent inhibition of the usual number of writhes observed in control animals in this study.

2.3. Control animals

Physiological salt solution (10 mL/kg) was injected intraperitoneally (i.p.) or given orally and control animals were run interspersed concurrently with the drug treatments, which prevented the controls being run on a single group of mice at one time (19.7 ± 0.28, $n = 92$ i.p.; 20.1 ± 0.33, $n = 12$ p.o.).

2.4. Protocol

Dose–response curves for the antinociceptive effect of diclofenac, ibuprofen, ketoprofen, meloxicam, metamizol, naproxen, nimesulide, paracetamol, parecoxib, and piroxicam were obtained using at least six animals at each of at least four doses. The drugs were injected intraperitoneally. Dose–response curves were also obtained by the oral administration of paracetamol and diclofenac. A least-squares linear regression analysis of the log dose–response curves allowed the calculation of the dose that produced 50% of antinociception (ED_{50}) for each drug. A dose–response curve was also obtained by the intraperitoneal coadministration of paracetamol with each NSAID in fixed ratio combinations of fractions of their respective ED_{50} values: 1/2, 1/4, 1/8, 1/16 (ratio values given in Table 2). A dose–response curve for the combination of paracetamol and diclofenac administered orally by gavage was also obtained with the same scheme. Isobolographic analysis was used to determine drug interactions. The method has been described previously in detail (Miranda et al., 2002). 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 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 was calculated as experimental ED_{50}/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 (Tallarida, 2001).

2.5. Drugs

The drugs were freshly dissolved in a physiological salt solution. Paracetamol was provided by Bristol-Myers-Squibb, France. The NSAIDs were provided by local pharmaceutical companies: diclofenac by Novartis Chile S.A., ibuprofen by Laboratorio Chile S.A., ketoprofen by Rhone-Poulenc Rorer, meloxicam and naproxen by Laboratorios Saval S.A., metamizol by Sanderson S.A., nimesulide by Grünenthal Chilena Ltda, parecoxib and piroxicam by Pfizer Chile. Doses were expressed on the basis of the salts.

2.6. Statistical analysis

Results are presented as means ± SEM 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.), based on Tallarida (2000). The statistical difference between theoretical and experimental

values was assessed by Student's t test for independent means and P values less than 0.05 ($P < 0.05$) were considered significant.

3. Results

3.1. Antinociception induced by paracetamol and NSAIDs

The i.p. administration of paracetamol, diclofenac, ibuprofen, ketoprofen, meloxicam, metamizol, naproxen, nimesulide, parecoxib and piroxicam showed dose-dependent antinociceptive effects with different potencies in the abdominal constriction test of mice. In Fig. 1, data showing dose–response curves obtained for several NSAIDs injected intraperitoneally (A) and for diclofenac and paracetamol given by the oral route (p.o., B) are displayed as an example. The curves were statistically parallel. The ED_{50} values and 95% CL for the antinociceptive effects of i.p. paracetamol and NSAIDs are shown in Table 1.

3.2. Interactions between paracetamol and NSAIDs

The antinociceptive activity of the i.p. coadministration of fixed ratio combinations of ED_{50} fractions of each NSAID with paracetamol was assessed by calculating the ED_{50} of the mixtures from the corresponding dose–response curves.

The isobolographic analysis of all combinations of NSAIDs with paracetamol, administered i.p., resulted

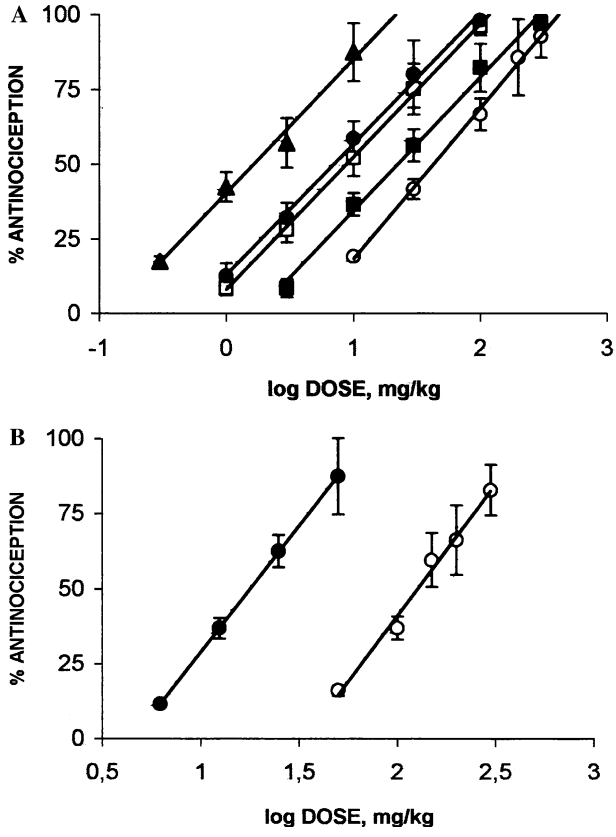

Fig. 1. Examples of dose–response curves for the antinociception induced by the intraperitoneal (A) and oral (B) administration of paracetamol (○), diclofenac (●), metamizol (■), parecoxib (▲) and piroxicam (□). Each point is the mean ± SEM of 6–8 animals.

Table 1
ED_{50} values and 95% CL for the antinociceptive effect of NSAIDs in the writhing test of mice

Drugs	ED_{50} mg/kg i.p.	CL	ED_{50} mg/kg p.o.	CL
Ibuprofen	0.8	0.12–6.1		
Parecoxib	1.6	1.0–2.6		
Meloxicam	6.5	4.9–8.4		
Nimesulide	7.6	5.8–9.8		
Diclofenac	8.1	5.7–10.8	17.9	17.6–18.5
Piroxicam	8.5	6.4–11.2		
Metamizol	28.5	21.8–37.6		
Ketoprofen	30.3	25.5–36.1		
Naproxen	46.4	35.7–60.5		
Paracetamol	49.4	33.4–59.1	127.2	112.2–143.6

Values are ranked in descending order of potency.

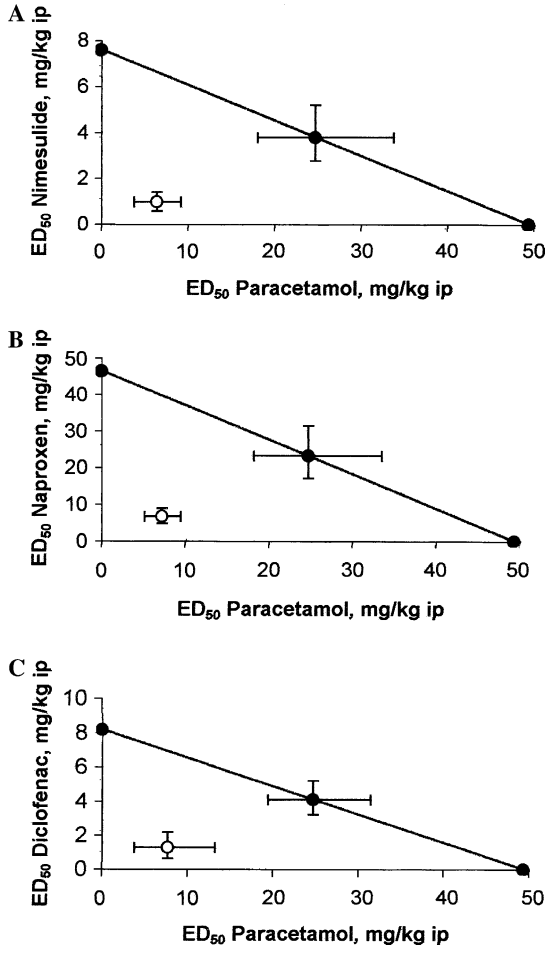

Fig. 2. Isobolograms for the intraperitoneal administration of the combinations nimesulide/paracetamol (A), naproxen/paracetamol (B) and diclofenac/paracetamol (C). Filled circles correspond to the theoretical ED_{50} with 95% CL and open circles correspond to the experimental ED_{50} with 95% CL.

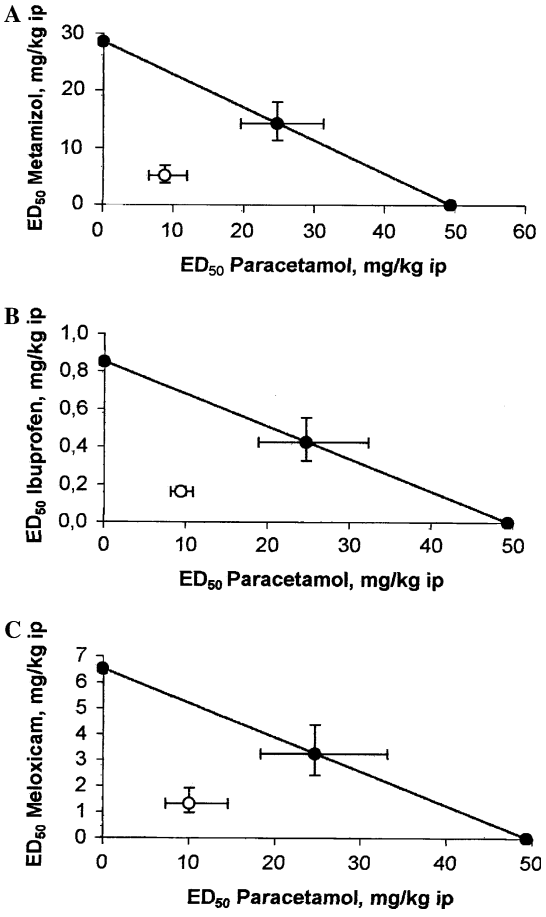

Fig. 3. Isobolograms for the intraperitoneal administration of the combinations metamizol/paracetamol (A), ibuprofen/paracetamol (B) and meloxicam/paracetamol (C). Symbols as in Fig. 2.

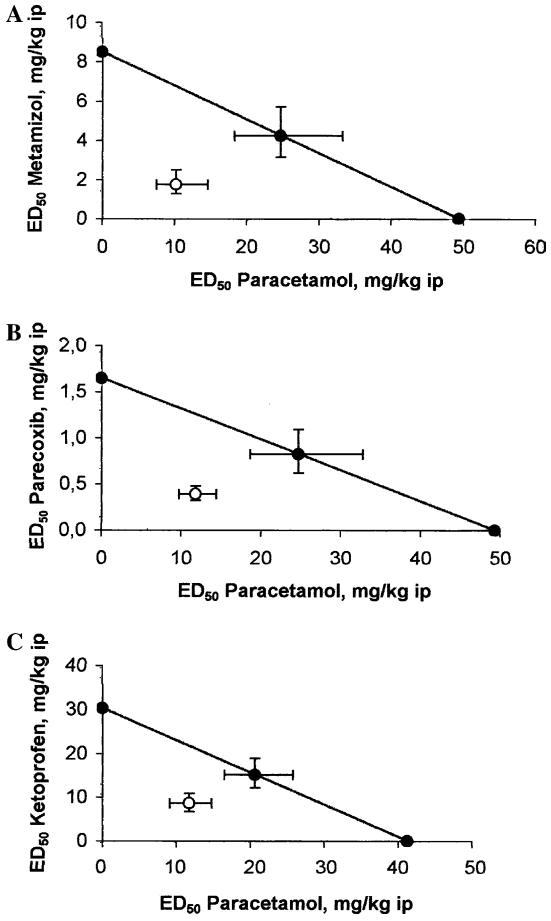

Fig. 4. Isobolograms for the intraperitoneal administration of the combinations piroxicam/paracetamol (A), parecoxib/paracetamol (B) and ketoprofen/paracetamol (C). Symbols as in Fig. 2.

in synergistic interactions of different magnitude, as can be seen in Figs. 2–4. The synergy was also present when the drug combination was administered orally (Fig. 5). Table 2 shows the theoretical additive and the experimental observed ED_{50} values for the combinations, with their 95% CL and their fixed ratios. Furthermore, the interaction index values of the i.p. combinations demonstrated the following rank of potencies: nimesulide/paracetamol > naproxen/paracetamol > diclofenac/paracetamol > metamizol/paracetamol > ibuprofen/paracetamol > meloxicam/paracetamol > piroxicam/paracetamol > parecoxib/paracetamol > ketoprofen/paracetamol (Table 3).

4. Discussion

The intraperitoneal coadministration of paracetamol with the following NSAIDs, diclofenac, ibuprofen, ketoprofen, meloxicam, metamizol, naproxen, nimesulide, parecoxib and piroxicam, produced a dose-dependent antinociceptive effect in the chemical viscero-somatic assay of the acetic acid abdominal constriction test. The oral administration of paracetamol and diclofenac showed similar results. The parallelism of the dose–response curves is consistent with a common mechanism of action. These results confirm previous findings in which either paracetamol or the above-mentioned NSAIDs showed antinociceptive activity in this algesiometric test (Miranda et al., 2001, 2002, 2003; Pinardi et al., 2001; Botting, 2003; Miranda and Pinardi, 2004).

Every combination tested showed a synergic interaction. The differences in the magnitude of the observed

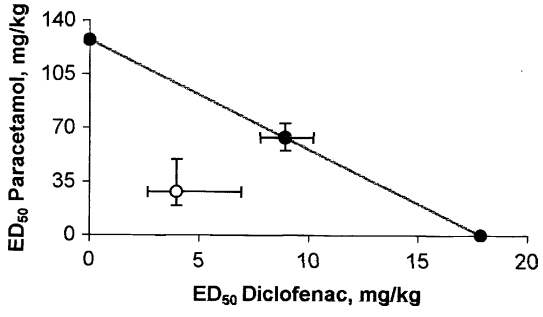

Fig. 5. Isobologram for the oral administration of the combination diclofenac/paracetamol. Interaction index = 0.445. Symbols as in Fig. 2.

Table 2
Theoretical and experimental ED_{50} values with 95% CL and ratios for combinations of NSAIDs with paracetamol (PARA) in the writhing test of mice

Combinations	ED_{50} (95% CL) mg/kg i.p.		Ratio NSAID:PARA
	Theoretical	Experimental	
Naproxen/paracetamol	47.9 (35.3–64.9)	13.9* (9.9–18.3)	1:1.06
Metamizol/paracetamol	38.9 (30.7–49.3)	13.8* (10.3–18.8)	1:1.73
Piroxicam/paracetamol	28.9 (21.4–39.0)	11.9* (8.8–17.1)	1:5.81
Diclofenac/paracetamol	28.8 (20.6–40.1)	7.4* (4.4–15.4)	1:6.03
Nimesulide/paracetamol	28.5 (20.8–38.9)	7.4* (4.4–10.6)	1:6.50
Meloxicam/paracetamol	27.9 (20.8–37.5)	11.4* (8.3–16.5)	1:7.60
Parecoxib/paracetamol	26.5 (19.2–33.8)	12.2* (10.0–14.8)	1:30.1
Ketoprofen/paracetamol	39.3 (33.5–47.4)	20.4* (15.8–25.6)	1:31.6
Ibuprofen/paracetamol	25.1 (19.2–32.8)	9.6* (8.3–11.1)	1:58.1
Combinations	ED_{50} (95% CL) mg/kg p.o.		Ratio NSAID:PARA
	Theoretical	Experimental	
Diclofenac/paracetamol	72.6 (63.3–83.1)	32.3* (21.6–53.3)	1:7.11

* $P < 0.05$.

interactions with the different NSAIDs, expressed by the interaction index, may be related with their COX selectivity, the potency of COX inhibition, pharmacokinetic properties or with additional mechanisms of action. Compared to the salicylates, NSAIDs are highly lipophilic substances able to cross the blood–brain barrier (Bannwarth et al., 1989; Mehanna, 2003) and this factor does not seem to contribute significantly to the interaction. There is no evident relationship between NSAIDs ED_{50} values (Table 1) reflecting relative potency and the interaction index. COX-2 selectivity (parecoxib > meloxicam > nimesulide, Warner and Mitchell, 2004) may be inversely related to the interaction index (Table 3), but this apparent relationship needs further studies. Additional mechanisms of action other than COX inhibition may strongly influence the interaction.

NSAIDs are eliminated primarily by hepatic glucuronidation and an inverse relationship may exist between glucuronidation and NSAIDs' efficacy, since an increase in the metabolism of these drugs results in a decrease of their pharmacological effect. The relative rates of NSAIDs glucuronidation are ketoprofen > ibuprofen > diclofenac > naproxen (Kuehl et al., 2005). This order of metabolic inactivation seems to be inversely related with the strength of the interaction index observed, since ketoprofen has the higher value and naproxen the lowest (Table 3).

Table 3
Interaction index (I.I.) of the combinations of NSAIDs with Paracetamol in the writhing test

Combination	Interaction index i.p.
Nimesulide/paracetamol	0.280
Naproxen/paracetamol	0.291
Diclofenac/paracetamol	0.311
Metamizol/paracetamol	0.356
Ibuprofen/paracetamol	0.380
Meloxicam/paracetamol	0.408
Piroxicam/paracetamol	0.413
Parecoxib/paracetamol	0.477
Ketoprofen/paracetamol	0.511
Combination	Interaction index p.o.
Diclofenac/paracetamol	0.445

Interaction index values i.p. are listed in ascending order. Lower values indicate higher potency of the combinations.

The findings of the present work are important, because they are concordant with several clinical studies in which, in different surgical procedures, the combinations of paracetamol with ketoprofen or diclofenac were associated with lower pain scores than paracetamol alone (Hyllested et al., 2002). Clinical information comparing the degree of analgesia induced by paracetamol/NSAIDs combinations versus that induced by the NSAIDs alone, however sparse the available data might be, suggests that standard doses of paracetamol enhance the analgesic efficacy when added to ketoprofen, diclofenac or naproxen (Seideman, 1993; Breivik et al., 1999).

The analgesic effect of NSAIDs may not simply reflect a common mechanism of action, namely inhibition of prostaglandin biosynthesis (Vane et al., 1998; Smith et al., 2000; Chandrasekharan et al., 2002; Warner and Mitchell, 2004). Additional mechanisms have been suggested for the antinociceptive effect of different NSAIDs (Granados-Soto et al., 1995; Cashman, 1996; Papworth et al., 1999; Miranda et al., 2001). It is interesting to note that COX-3, a splice variant of COX-1, was considered to be the central site of action of paracetamol, but this selective interaction is unlikely to be clinically relevant (Graham and Scott, 2005) and the mechanism of paracetamol-induced analgesia in some way must affect COX-1 and/or COX-2

(Schwab et al., 2003). The study of Koppert et al. (2004) supports the assumption that additional mechanisms are involved in the antihyperalgesic action of paracetamol, since this drug is a nonspecific and weak inhibitor of COX-3. Recent data from Lucas et al. (2005) suggest that paracetamol interferes only with the oxidation state of COX-3. A mechanism of action has been recently suggested for paracetamol-induced analgesia in the mouse abdominal constriction test through a central action paralleled by a reduction in brain PGE_2 concentrations (Cashman, 1996; Botting and Ayoub, 2005).

Other findings suggested that paracetamol elicits the activation of one or more endogenous opioid pathways (Raffa et al., 2000, 2004). Furthermore, paracetamol may stimulate the activity of descending 5-HT pathways that inhibit nociceptive signal transmission in the spinal cord (Bonnefont et al., 2003). Taken together, these observations suggest that several mechanisms are probably implicated in the antinociceptive activities of paracetamol and of NSAIDs, many of them at central levels, all of which contribute to the synergy of the combinations, and justify the differences in the interaction index of paracetamol/NSAID mixtures observed in the present work. The mechanisms responsible for the synergism in the analgesic activity of paracetamol/NSAIDs combinations are not clear; however, according to the information in the literature, different systems are partially involved and further experiments are required to completely characterize the pharmacological basis of the synergic effect.

In conclusion, the data of the present study demonstrated that paracetamol combined with NSAIDs produces a supra-additive or synergic analgesic effect. It may be noted that the doses of paracetamol and NSAIDs are very small and if they are compared with those referred in the literature, it is possible to suggest that the combinations of paracetamol and NSAIDs will be effective for the clinical treatment of pain. In addition, it is demonstrated that the effect of the combinations paracetamol/NSAIDs is superior to that of either component alone. Therefore, these mixtures are a viable alternative to clinical pain management, especially because the low doses of the components may be a potential index of lower incidence of adverse effects.

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