Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 484 (2004) 157–165
www.elsevier.com/locate/ejphar

Analysis of the analgesic interactions between ketorolac and tramadol during arthritic nociception in rat

Francisco Javier López-Muñoz[a,*], Ma. Irene Díaz-Reval[b], José Antonio Terrón[c], Myrna Déciga Campos[a]

[a] *Laboratorio No. 7, "Dolor y Analgesia" del Departamento de Farmacobiología, CINVESTAV-IPN, Calz. de los Tenorios No. 235 Col. Granjas Coapa, Deleg. Tlálpan, Mexico, DF, CP 14330, Mexico*
[b] *Centro Universitario de Investigaciones Biomédicas de la Universidad de Colima, Mexico*
[c] *Sección Externa de Farmacología, CINVESTAV-IPN, Mexico, DF, Mexico*

Received 2 September 2003; received in revised form 27 October 2003; accepted 4 November 2003

Abstract

The potential advantage of using combination therapy is that analgesia can be maximized while minimizing the incidence of adverse effects. In order to assess a possible synergistic antinociceptive interactions, the antinociceptive effects of ketorolac tromethamine, p.o., a nonsteroidal anti-inflammatory drug (NSAID), and tramadol hydrochloride, p.o., an atypical opioid analgesic, administered either separately or in combination, were determined using a rat model of arthritic pain. The data were interpreted using the surface of synergistic interaction (SSI) analysis and an isobolographic analysis to establish the nature of the interaction. The surface of synergistic interaction was calculated from the total antinociceptive effect produced by the combination after subtraction of the antinociceptive effect produced by each individual drug. Female rats received orally ketorolac alone (0.18, 0.32, 0.56, 1.0, 1.78, 3.16, and 5.62 mg/kg), tramadol alone (3.16, 5.62, 10.0, 17.78, 31.62, 56.23, and 100.0 mg/kg), or 24 different combinations of ketorolac plus tramadol. Ten combinations exhibited various degrees of potentiation of antinociceptive effects (17.78 mg/kg tramadol with either 0.18, 0.32, or 0.56 mg/kg ketorolac; 10.0 mg/kg tramadol with either 0.18, 0.32, 0.56, or 1.8 mg/kg ketorolac; 5.6 mg/kg tramadol with either 0.32 or 0.56 mg/kg ketorolac; and 3.16 mg/kg tramadol with 0.32 mg/kg ketorolac), whereas the other 14 combinations showed additive antinociceptive effects. Three combinations of ketorolac + tramadol (1.0 + 17.78, 1.78 + 10, and 1.78 + 17.78, mg/kg respectively) produced the maximum antinociceptive effects, and two combinations (0.32 + 10.0 and 0.56 + 10.0 mg/kg, respectively) presented effects of high potentiation ($P < 0.001$). This combination caused gastric injuries less severe than those observed with indomethacin. The synergistic antinociceptive effects of ketorolac and tramadol were important and suggest that combinations with these drugs may have clinical utility in pain therapy.

Keywords: Inflammatory pain; Cyclooxygenase; Ketorolac; Tramadol; Synergism

1. Introduction

Analgesic agents of the opioid group generally possess a high analgesic efficacy, but they are also endowed with undesirable properties. Many, if not all, of these adverse effects are dose-dependent, and there are many other important factors, such as the pharmacokinetic problems (for instance, P450) and polymorphism of the gene encoding the μ-opioid receptor, that have been shown to be greatly associated with the clinical effects of opioid drugs. In the treatment of clinical pain, the choice of a specific analgesic drug is, in general, made on the basis of the type of pain. The opioid analgesic drugs remain the most effective therapy available for the treatment of moderate to severe pain; however, the problems arising from unwanted side effects persist. Therefore, the combinations of opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to control postoperative pain (Wideman et al., 1999; Picard et al., 1997). The potential advantage of using combination therapy is that analgesic effects can be maximised while the incidence of adverse side effects is minimized (Picard et al., 1997). Therefore, using combinations of medications that offer analgesic synergism should allow a reduction in required dosage and decrease the incidence of adverse effects (Wei-wu et al., 1999). One mechanism of action of NSAIDs involve suppression of the synthesis of

* Corresponding author. Tel.: +52-55-50612851; fax: +52-55-50612863.
E-mail address: flopezm@prodigy.net.mx (F.J. López-Muñoz).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.11.005

prostaglandins (Ferreira and Vane, 1974; Lorenzetti and Ferreira, 1985) and the mediation of the arginine–nitric oxide–cGMP pathway (Duarte et al., 1992). More recent evidence suggests that NSAID may also have direct central effects (Carlsson et al., 1988; Tortorici and Vanegas, 1994). Despite their ability to reduce pain and inflammation, NSAIDs cause a wide variety of reported adverse events, the most clinically important of which are upper gastrointestinal side effects, such as dyspepsia, peptic ulceration haemorrhage, and perforation, leading to death in some patients (Griffin, 1998).

Ketorolac tromethamine is an NSAIDs, available in both oral and parenteral forms, that possesses significant analgesic potency. Its analgesic efficacy has been studied extensively for the treatment of moderate to severe pain in many clinical settings. Oral ketorolac has been shown to provide analgesia that is the same or better than aspirin, acetaminophen, and dextropropoxyphene with acetaminophen (Catapano, 1996), and equal analgesia to most other commonly available oral analgesics, including ketoprofen and diclofenac (Forrest et al., 2002). Tramadol hydrochloride is a synthetic analogue of codeine that binds to μ-opiate receptors and inhibits norepinephrine and serotonin reuptake. It is rapidly and extensively absorbed after oral doses and is metabolized in the liver (Lewis and Han, 1997).

On the other hand, while clinical studies with NSAIDs and opioids suggest an additive or possibly synergistic interaction, few quantitative studies to establish the antinociceptive interaction have been conducted. Quantifying an antinociceptive synergistic effect presents practical and ethical limitations in human subjects, but adequate animal models of nociception have been described. There are combinations of opioids and NSAIDs that have positive synergistic interactions (Wideman et al., 1999; Picard et al., 1997; Wei-wu et al., 1999), but only few of these have been analysed in preclinical models (Maves et al., 1994; Sandrini et al., 1998, 1999; Taylor et al., 1998). Our group has addressed the analysis and evaluation of interactions between opioids and NSAIDs (López-Muñoz et al., 1993a, 1994; López-Muñoz, 1994; Salazar et al., 1995; Déciga-Campos et al., 2003). The purpose of the present work was to investigate the antinociceptive effect of ketorolac and tramadol by administration alone or in combination, using the pain-induced functional impairment (PIFIR) model in the rat, an animal model of arthritic pain (López-Muñoz et al., 1993b).

2. Materials and methods

2.1. Animals

Female Wistar rats [Crl (WI) BR], weighing 180–200 g, were used in this study. Food was withheld 12 h before the experiments, with free access to water. All experimental procedures followed the recommendations of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Covino et al., 1980) and the Guidelines on Ethical Standards for Investigations of Experimental Pain in Animals (Zimmermann, 1983), and were carried out according to a protocol approved by the local Animal Ethics Committee. The number of experimental animals was kept to a minimum, and animals were housed in a climate- and light-controlled room with a 12-h light/dark cycle.

2.2. Drugs

Uric acid (Sigma, St. Louis, MO, USA) was suspended in mineral oil; ketorolac tromethamine and tramadol hydrochloride were obtained from Laboratories RIMSA (Mexico City, Mexico). Ketorolac, tramadol, and indomethacin (Sigma) were dissolved in 0.5% carboxymethylcellulose and were administered orally.

2.3. Measurement of antinociceptive activity

Antinociceptive activity was assessed using the PIFIR model, which has been described in detail (López-Muñoz et al., 1993b). The animals were anaesthesised with ether in an anaesthesia chamber (Pyrex glass dryer saturated with ether vapor). Nociception was induced by an intra-articular (i.a.) injection of 0.05 ml of 30% uric acid suspended in mineral oil in the knee joint of the right hind limb. The suspension was prepared by grinding 3.0 g of uric acid with 10 ml of mineral oil in a glass mortar and pestle (Pyrex). The intra-articular injection was performed through the patellar ligament using a 1-ml glass syringe (Becton Dickinson LTDA, Brazil) with a 24-gauge needle of 5 mm. Immediately afterwards, an electrode was attached to the plantar surface of each hind paw between the plantar pads. The rats were allowed to recover from anaesthesia and were then placed on a stainless steel cylinder of 30 cm diameter, which was rotated at 4 rpm, forcing the rats to walk for periods of 2 min every 30 min for 6.5 h. Training periods were not necessary because the rats learned in the first minutes. The time of contact between each electrode on the limbs of the rat and the cylinder was recorded with a computer, this being the variable measured. When the electrode placed on the animal's paw made contact with the cylinder floor, a circuit was closed and the time that the circuit remained closed was recorded. After uric acid injection, the rats developed progressive dysfunction of the injured limb. The time of contact of the injured hind limb reached a zero value 2.5 h after the injection of uric acid; at this time, ketorolac and tramadol were administered either alone or in combination. This time was considered as time zero for measurement of antinociceptive effects, and these effects were measured every 30 min for the next 4 h. This permitted determination of the time course of the antinociceptive effects in the same animal. Antinociception was estimated as recovery of the time of contact. The data are expressed as the functionality index percent (FI%; i.e., the time of contact of the injected foot divided by the time of contact of the control

left foot multiplied by 100). For the purpose of this study, inducing nociception in the experimental animals was unavoidable. However, care was taken to avoid unnecessary suffering. All experiments were performed between 7:00 a.m. and 2:00 p.m.

2.4. Study design

The antinociceptive effects produced by ketorolac and tramadol given either individually or in combination were studied. First, each dose of ketorolac (0.18, 0.32, 0.56, 1.0, 1.78, 3.16, or 5.62 mg/kg) or tramadol (3.16, 5.62, 10.0, 17.78, 31.62, 56.23, or 100.0 mg/kg) was given to six animals to obtain the corresponding dose–response curves, and the doses of ketorolac (0.10, 0.18, 0.32, 0.56, 1.0, or 1.78 mg/kg) and tramadol (3.16, 5.62, 10.0, or 17.78 mg/kg) were then combined to analyse possible synergistic interactions (24 combinations in total). At the end of the experiment, the rats were euthanized.

2.5. Measurement of gastrointestinal side effects

Female Wistar rats (150–180 g of body weight) were fasted 24 h before the experiments. Indomethacin (20 mg/kg) was given to produce 100% gastric ulcers (Lee et al., 1971; Déciga-Campos et al., 2003). Ketorolac (1.0 mg/kg), tramadol (17.8 mg/kg), vehicle (carboxymethylcellulose of 0.5%), and the combination of ketorolac plus tramadol (1.0 and 17.8 mg/kg, respectively) were administered orally at the same time to five groups (six rats each). About 2.5 h later, all the groups received a second administration of the same doses. Stomachs were examined 5 h after the first treatment as follows: the animals were killed and the stomachs were removed, opened along the smaller curvature, gently rinsed under formol (2%), and examined. The severity of gastric lesions induced by the drug treatments was calculated as the ratio between the number of lesions (stomach ulcer or erosion) caused by a given treatment and the number of lesions produced by indomethacin (100%). This was considered to reflect drug-induced adverse effects. The sum of the area of all ulcers in the corpus for each animal was calculated and served as the ulcer index. Gastric injury percent was calculated as:

$$\%\ \text{Gastric Injury} = (\text{UIT}/\text{UII}) \times 100$$

where UIT is the ulcer index in drug test (mm^2), and UII is the ulcer index in indomethacin test (mm^2).

2.6. Data presentation and statistical evaluation

Data in the text, tables, and figures are expressed as the FI%. Curves for FI% vs. time were made for each treatment and the corresponding time course was obtained. Antinociception was estimated as the recovery of the FI%. The cumulative antinociceptive effect during the whole observation period (4 h) was determined as the area under the curve (AUC) of the time course to obtain the dose–response curve and to analyse the whole antinociceptive effect elicited by the analgesic agent, either alone or in combination.

The synergism between ketorolac and tramadol was calculated with surface of synergistic interaction (SSI) analysis (López-Muñoz, 1994) and an isobolographic method (Tallarida et al., 1989). The AUC was calculated for each of the drug combinations and for each of the components. On the basis of the addition of the effects of the individual component drugs (Seegers et al., 1981), an AUC equivalent to the sum was expected. If the sum of the corresponding individual AUCs was higher than the theoretical sum, the result was considered to show potentiation; if it was similar to the theoretical sum, it was considered to show an additive antinociceptive effect. The AUC was obtained by the trapezoidal rule (Rowland and Tozer, 1989). All values for each treatment are mean ± S.E.M for six animals. The AUC values for drug combinations were compared with the expected value using Student's *t* test. The AUC values obtained from the antinociceptive effects produced by either ketorolac or tramadol (assayed separately) were compared with the AUC value obtained from the corresponding combination by analysis of variance (ANOVA) and Dunnett's test. The gastrointestinal side effects produced by either ketorolac or tramadol (assayed either separately or in combination) were compared with the gastrointestinal side effects obtained from indomethacin by ANOVA and Dunnett's test. $P < 0.05$ was considered statistically significant ([a]).

The doses producing 50% of the maximum possible effect (ED_{50}) of each drug were calculated by performing a linear regression analysis of the linear portion of the dose–response curves. The isobologram was constructed using ED_{50} when the drugs were given alone or in combination. To perform the isobolographic analysis, ketorolac and tramadol were administered in combination as fixed ratios of the equieffective ED_{50} dose for each drug (ketorolac:tramadol = 1:1). The ED_{50} values (± S.E.M.) for ketorolac and tramadol alone were plotted on the *x*- and *y*-axes, respectively, and the theoretical additive point was calculated according to Tallarida et al. (1989). From the dose–response curve of the combined drugs, the ED_{50} value of the total dose of the combination was calculated. Statistical significance of the difference between the theoretical additive point and the experimentally derived ED_{50} value was evaluated using Student's *t* test. An experimental ED_{50} significantly less than the theoretical additive ED_{50} ($P < 0.05$) was considered to indicate a synergistic interaction between ketorolac and tramadol.

3. Results

3.1. Effect of uric acid and vehicles

Uric acid induced complete dysfunction of the right hind limb corresponding to a FI% value of zero in 2.5 h. This

dysfunction was maintained throughout the entire experimental period, which lasted another 4 h. The rats that received vehicle (methylcellulose 0.5%) did not show any significant recovery of the FI% during the observation period. At the doses used, ketorolac (0.18–5.62 mg/kg) and tramadol (3.16–56.23 mg/kg) did not affect the walking ability of the rats during the period of evaluation, as compared with that of the vehicle-treated rats (data not shown). Tramadol, 100 mg/kg, produced side effects in 50% of the animals: sedation, euphoria, dizziness, and effect in the walking ability of the rats.

3.2. Antinociceptive effects of drugs assayed individually

Fig. 1 shows the dose–response curves for ketorolac and tramadol. Both drugs increased AUC in a dose-dependent manner but displayed different efficacy (i.e., ketorolac produced the maximum effect). Thus, ketorolac (3.16 mg/kg) showed a great antinociceptive efficacy of 295.8 ± 14.9 au and tramadol (56.23 mg/kg) showed 207.7 ± 24.7 au. The ED_{50} values for the drugs indicate that there were significant differences in their antinociceptive potencies: ketorolac (ED_{50} = 0.86 ± 0.10 mg/kg) was more potent than tramadol (ED_{50} = 44.29 ± 0.06 mg/kg). There were no adverse effects with the doses used. The antinociceptive effect shown by 100 mg/kg tramadol (236.7 ± 11.0 au) is not included in Fig. 1 because it produced side effects in the rats.

3.3. Antinociceptive effects of the drug combinations

Figs. 2–4 depict the antinociceptive effect from the 24 combinations on three-dimensional graphs. These were constructed using the mean from six animals for each dose either alone or in combination. The maximal antinociceptive effect attainable from several ketorolac + tramadol combinations (1.78 + 17.78 mg/kg, respectively; see Fig. 2) was 372.7 ± 15.6 au. Statistical analysis of data from Fig. 2 indicates an interaction between ketorolac and tramadol

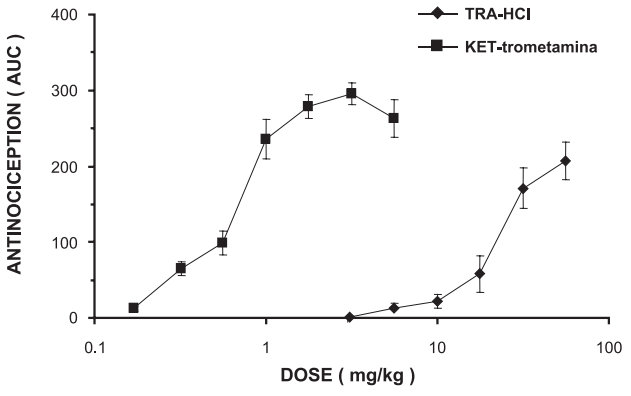

Fig. 1. Oral antinociceptive effect of ketorolac and tramadol in the pain-induced functional impairment model. The response is expressed on the *y*-axis as the AUC of the functionality index over the 4-h observation period (% h). Data are expressed as means ± S.E.M. for six animals.

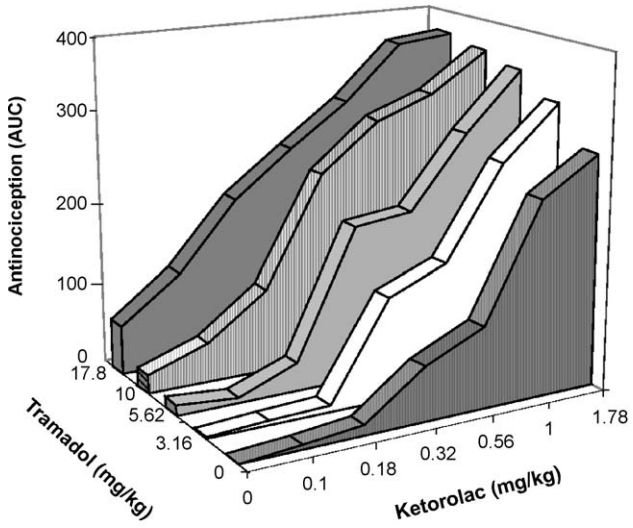

Fig. 2. Antinociceptive effects obtained with ketorolac and tramadol either alone or in combination. The *y*-axis represents the AUC of the time course, the *x*-axis depicts the doses (mg/kg) of tramadol administered simultaneously with ketorolac, and the *z*-axis depicts the doses (mg/kg) of ketorolac used to obtain the dose–response curves (DRC). The combination of ketorolac (1.78 mg/kg) + tramadol (17.78 mg/kg) showed the greatest antinociceptive effect. Each point represents the mean of six experiments; there is an interaction between ketorolac and tramadol ($P < 0.05$).

($P < 0.05$), whereas there were no antagonistic effects of the combinations tested.

Fig. 3 was produced with the objective of discerning additive from potentiation effects. This graph was calculated from the total antinociceptive effect produced by the combinations after subtraction of the antinociceptive effect produced by each component alone. Results higher than level "0" were considered to indicate potentiation, whereas those at level "0" were considered to indicate addition. Although this type of plot allows antagonistic antinociceptive effects to be observed, these were not obtained in the present study. Likewise, 14 combinations of ketorolac + tramadol produced additive antinociceptive effects, and 10 produced potentiation with 95% confidence limits ($P < 0.05$) ([a] in Fig. 3). These combinations were: 17.78 mg/kg tramadol with either 0.18, 0.32, or 0.56 mg/kg ketorolac; 10.0 mg/kg tramadol with either 0.18, 0.32, 0.56, or 1.78 mg/kg ketorolac; 5.62 mg/kg tramadol with either 0.32 or 0.56 mg/kg ketorolac; and 3.16 mg/kg tramadol with 0.32 mg/kg ketorolac. In order to obtain the surface of synergistic interaction for the combinations ketorolac + tramadol, all the points of interaction from Fig. 3 were unified in a plane. The result is the surface of synergistic interaction of these analgesic drugs shown in Fig. 4. Using this graph, it is easy to visualise the drug interactions of ketorolac + tramadol (i.e., addition or potentiation). For example, 10 combinations of ketorolac + tramadol displayed various degrees of potentiation of antinociceptive effects, but two combinations of ketorolac + tramadol showed effects of high potentiation (0.32 + 10 and 0.56 + 10 mg/kg, respectively) (see Table 1).

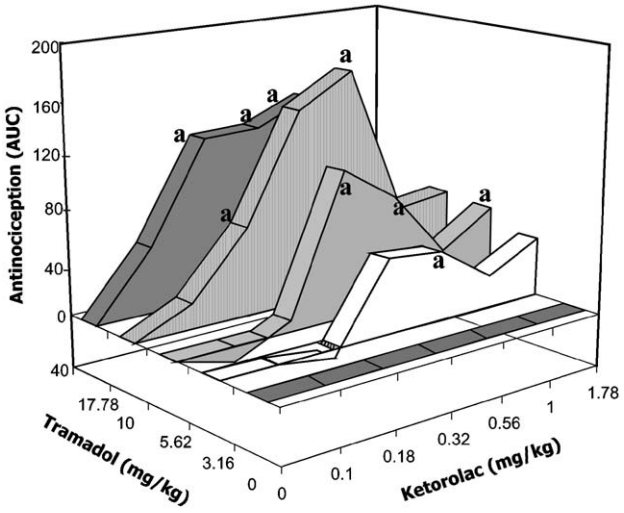

Fig. 3. The antinociceptive effects produced by the different combinations of ketorolac and tramadol after subtracting the individual effects. The axes are the same as those in Fig. 2. Ten results correspond to potentiation of antinociceptive effects ($^{a}P<0.05$), whereas the other 14 combinations represent addition of antinociceptive effect. The combination of ketorolac + tramadol (0.56 + 10.0 mg/kg) produced higher potentiation ($P<0.001$). Each interaction is represented by the mean for six animals.

Tramadol, at a dose of 10 mg/kg, yielded an AUC of 22.5 ± 9.3 au and ketorolac, at the dose of 0.32 mg/kg, rendered an AUC of 65.0 ± 9.6 au; however, the combination of ketorolac + tramadol (10.0 + 0.32 mg/kg) yielded an AUC of 233.5 ± 25.8 au, which is higher than the expected AUC resulting from the sum of the individual values (i.e., 87.5 au) ($P<0.001$). The analysis of the E_{max} from the corresponding time course curves showed an increase in the values obtained from the combination (86.6 ± 6.3%), which were higher than the corresponding values (ketorolac 33.7 ± 8.7% and tramadol 17.1 ± 9.1%) obtained from the arithmetic sum (50.8%). Other examples of potentiation with ketorolac + tramadol are shown in Table 1.

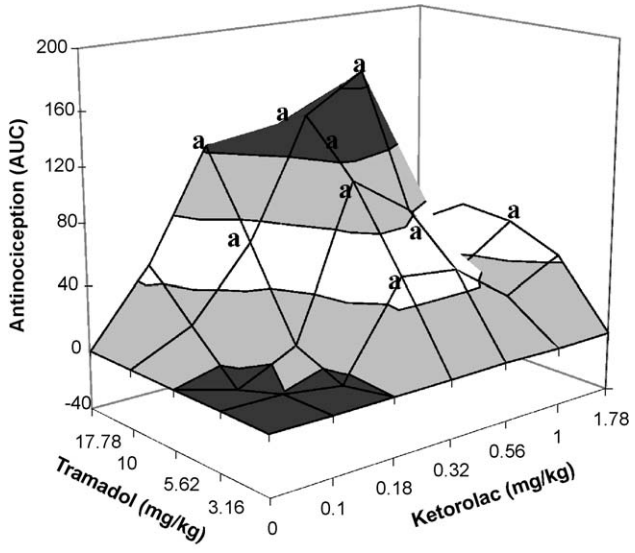

Fig. 4. Determination of the SSI for the combination of ketorolac + tramadol. All the points of synergism from Fig. 3 have been joined by a plane. On this graph, the axes are the same as in Fig. 2. The doses producing either potentiation or addition when coadministered could easily be determined. Ten combinations showed various degrees of potentiation (a), while others exhibited only additive antinociceptive effects.

Table 1
Comparison of the antinociceptive effects expressed as AUC and FI% produced by some combinations and those produced by the maximal dose of each analgesic drug (ketorolac or tramadol)

Treatment	Dose (mg/kg)	AUC[a] (au)	Time course curve[b]		
			E_{max} (FI%)	TE_{max}[c] (h)	$E_{4\ h}$ (4 h after)[d] (FI%)
KET	1.0	235.6 ± 25.8	67.6 ± 9.4	0.75	49.7 ± 10.1
TRA	17.78	58.2 ± 23.7	37.0 ± 19.5	0.75	4.9 ± 3.9
KET + TRA(1)		366.0 ± 12.2	102.4 ± 9.0	0.50	94.0 ± 4.2
KET	1.78	279.0 ± 15.5	86.4 ± 5.9	2.00	65.2 ± 7.5
TRA	10.0	22.5 ± 9.3	17.1 ± 9.1	0.75	1.6 ± 1.6
KET + TRA(2)		363.4 ± 13.6[e]	100.2 ± 5.5	0.75	91.3 ± 9.0
KET	1.78	279.0 ± 15.5	86.4 ± 5.9	2.00	65.2 ± 7.5
TRA	17.78	58.2 ± 23.7	37.0 ± 19.5	0.75	4.9 ± 3.9
KET + TRA(3)		372.7 ± 15.6[e]	93.2 ± 5.3	0.50	95.2 ± 5.5
KET	0.32	65.0 ± 9.6	33.7 ± 8.7	0.75	12.1 ± 6.1
TRA	10.0	22.5 ± 9.3	17.1 ± 9.1	0.75	1.6 ± 1.6
KET + TRA(4)		233.5 ± 25.8[e]	86.6 ± 6.3	0.75	31.0 ± 9.9
KET	0.56	98.7 ± 15.5	51.4 ± 7.9	0.75	11.2 ± 5.5
TRA	10.0	22.5 ± 9.3	17.1 ± 9.1	0.75	1.6 ± 1.6
KET + TRA(5)		290.6 ± 27.7[e]	93.2 ± 7.2	0.50	48.9 ± 17.8
KET(6)	3.16	295.8 ± 14.9	75.1 ± 9.9	0.50	77.4 ± 6.8
TRA(7)	56.23	207.7 ± 24.7	65.1 ± 9.9	0.50	29.9 ± 11.7

(1)–(3): Combinations that produced the maximum antinociceptive effect; (4) and (5): combinations that produced high potentiation.

[a] Area under the curve of the time course or the whole antinociceptive effect shown for the analgesic drug during the 4-h period, either alone or in combination.

[b] Variables measured for curves of time course.

[c] Time to produce the maximal effect measured for curve of time course.

[d] This FI expresses the antinociceptive effect obtained exactly 4 h after administration; this is the last evaluation of antinociception in the experimental protocol.

[e] $P<0.01$.

The antinociceptive effects produced by the combinations that produced the maximum antinociceptive effect (1.78 mg/kg ketorolac + 17.78 mg/kg tramadol) and the combination that produced high potentiation (0.56 mg/kg ketorolac + 10.0 mg/kg tramadol) are shown in Fig. 5. As can be seen in Fig. 5A, the antinociception produced by ketorolac + tramadol (1.78 + 17.78 mg/kg) represented the maximum antinociceptive effect (which represents a total recovery) obtained with 372.7 ± 15.6 au, while ketorolac alone (1.78 mg/kg) showed an AUC of 279.0 ± 15.5 au and tramadol alone (17.78 mg/kg) produced 58.2 ± 23.7 au

only. This result was important if it is considered that the maximum ketorolac dose used (3.16 mg/kg) produced less antinociceptive effect: 295.8 ± 14.9 au. The combination depicted in Fig. 5B (0.56 mg/kg ketorolac + 10.0 mg/kg tramadol) merely represents a combination that produced the maximum potentiation of the antinociceptive effect (169.4% more AUC or whole antinociceptive effect than the sum of individual AUCs); likewise, both the time course and AUC obtained with this combination were higher ($P < 0.001$) than the respective values obtained with the sum of individual agents (121.2 au). The antinociception produced by ketorolac + tramadol (0.56 + 10.0 mg/kg) was 290.6 ± 27.7 au, while ketorolac alone (0.56 mg/kg) showed an AUC of 98.7 ± 15.5 au and tramadol alone (10.0 mg/kg) produced 22.5 ± 9.3 au only. A significant antinociceptive effect was obtained with the combination during all the observation periods (4 h).

Another approach for investigating the synergistic interaction between the two selected analgesic drugs is the isobolographic method (Tallarida et al., 1989). An isobologram showing the antinociceptive interaction of ketorolac and tramadol in the pain-induced functional impairment model in the rat is shown in Fig. 6. Horizontal and vertical bars indicate S.E.M. The oblique line between the *x*- and *y*-axes is the theoretical additive line. The point in the middle of this line is the theoretical additive point calculated from the separate ED_{50} values. The experimental point lies far below the additive line, indicating a significant synergism ($P < 0.05$).

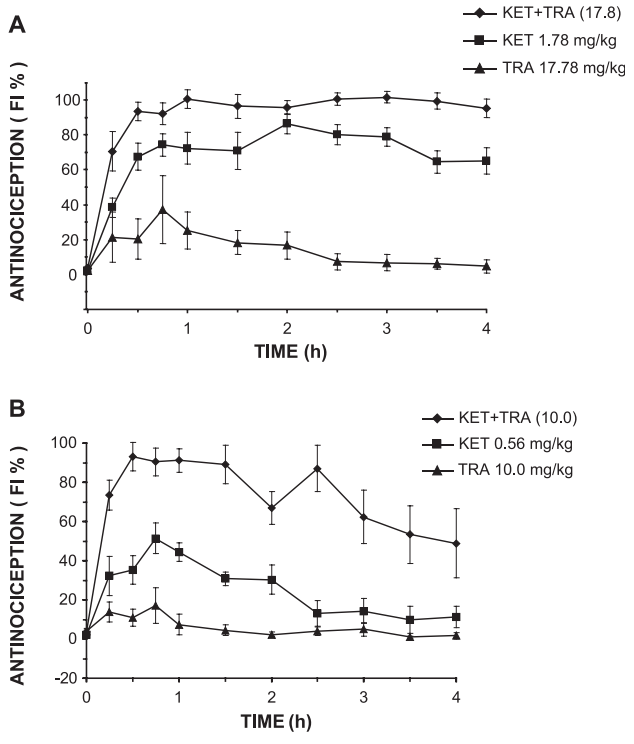

Fig. 5. Time courses of the combinations that produced (A) the maximum antinociceptive effect: 1.78 mg/kg ketorolac (■), 17.78 mg/kg tramadol (▲), and the combination of ketorolac + tramadol (1.78 + 17.78 mg/kg) (◆); and (B) high potentiation: 0.56 mg/kg ketorolac (■), 10.0 mg/kg tramadol (▲), and the combination of ketorolac + tramadol (0.56 + 10.0 mg/kg) (◆). This latter combination represents a clear example of potentiation of the antinociceptive effects; the AUC (290.6 ± 27.7 au) obtained with this combination was higher ($P < 0.001$) than the AUC obtained from the sum of the individual AUC (98.7 ± 15.5 + 22.5 ± 9.3 au). Data are expressed as the mean ± S.E.M. of six determinations.

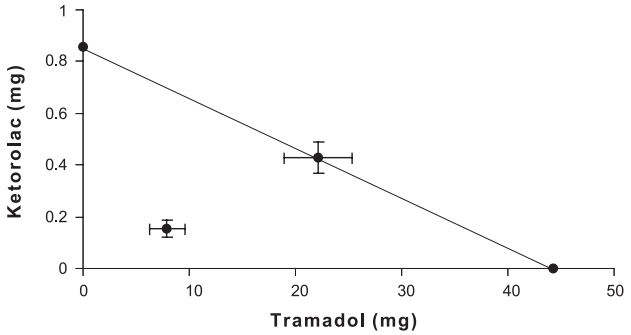

Fig. 6. Isobologram showing the antinociceptive interaction of ketorolac (ED_{50} = 0.86 ± 0.10 mg/kg) and tramadol (ED_{50} = 44.29 ± 0.06 mg/kg) in the PIFIR model. Horizontal and vertical bars indicate S.E.M. The oblique line between the *x*- and *y*-axes is the theoretical additive line. The point in the middle of this line is the theoretical additive point calculated from the separate ED_{50} values. The experimental point lies far below the additive line, indicating significant synergism ($P < 0.05$).

3.4. Measurement of gastrointestinal side effects

The administration of tramadol did not produce ulcers or erosions. Its adverse effects were similar to those of vehicle. However, ketorolac generated a lower area of ulcers (13.7 ± 2.7 mm^2) and less number of erosions (15.0 ± 4.9) than did indomethacin ($P < 0.05$), which was considered to be the most detrimental compound in terms of the number and severity of the lesions caused in the stomach (i.e., ulcers or erosions) (100%). The combination of ketorolac + tramadol generated less ulcers (16.9 ± 6.9 mm^2) and less number of erosions (5.0 ± 1.4) than did indomethacin ($P < 0.05$). Interestingly, the combination of ketorolac + tramadol decreased the generation of erosions ($P < 0.05$) and the ulcerations were similar to those of ketorolac alone (Table 2).

Table 2
Comparison of the gastrointestinal side effects expressed as percent of gastric injury produced by indomethacin (20 mg/kg), ketorolac (1 mg/kg), tramadol (17.8 mg/kg), and the combination of ketorolac and tramadol (1 and 17.8 mg/kg, respectively)

Treatment	Number of erosions	Ulcers[a] (mm^2)	Gastric injury[b] (%)
Vehicle	0	0	0
Indomethacin	30.0 ± 3.9	51.6 ± 3.4	100
Ketorolac	15.0 ± 4.9[c]	13.7 ± 2.7[c]	26.6
Tramadol	0	0	0
Ketorolac/tramadol	5.0 ± 1.4[c]	16.9 ± 6.9[c]	32.8

[a] Sum of the area of all ulcers.
[b] (Ulcer index in drug test/ulcer index in indomethacin test) × 100, where ulcer index is the sum of the area of all ulcers in the stomach.
[c] ANOVA (Dunnett's test), $P < 0.05$ with respect to indomethacin.

4. Discussion

Some reports have been published on analgesic combinations (López-Muñoz et al., 1993a, 1994; López-Muñoz, 1994; Salazar et al., 1995; Maves et al., 1994; Sandrini et al., 1998, 1999; Taylor et al., 1998; Déciga-Campos et al., 2003). Therefore, the focus of this study was to first examine the antinociceptive efficacy of either ketorolac or tramadol during arthritic pain, and, second, to quantitatively evaluate the analgesic interaction between ketorolac and tramadol. The clinical implications of this study are important, given the desire to maximize analgesia while minimizing adverse effects in a variety of situations in which arthritic pain is a major problem.

This is the first study analysing the effects of combinations of ketorolac and tramadol. The reason for using tramadol was that it is an atypical opioid, whereas ketorolac is a widely used analgesic in Mexico. The PIFIR model was used because it allows the evaluation of the time course of the antinociceptive effect in the same animal; furthermore, it does not generate conditioned learning and has high sensitivity (López-Muñoz et al., 1993b). The doses used for obtaining the dose–response curve of either ketorolac or tramadol alone were selected on an increasing 0.25 logarithmic units basis. The doses used for analysing the combinations (surface of synergistic interaction analysis) were selected from the respective dose–response curves. The 24 different associations were planned using six doses of the NSAID (almost all the doses of ketorolac were used) with four low doses of the morphine-like drug: tramadol. This allowed for the detection of the profile of antinociceptive interaction between the various combinations. The doses used to analyze the effects of the combinations were selected on the basis of their lack of adverse effects when administered alone. The surface of synergistic interaction analysis was applied to assess the antinociceptive effects produced by ketorolac and tramadol, either separately or in different combination ratios, which may allow the determination of the optimal ratio that produces the antinociceptive activity (i.e., maximum antinociceptive effect and optimal potentiation); the isobolographic method, on the other hand, was used to confirm the synergistic effect. The two methods used to assess synergistic interaction between antinociceptive compounds are excellent tools in pharmacology, but it should be highlighted that the purpose of the present study was not related with the comparison of the two methods to assess synergistic interaction between antinociceptive compounds. Our group has used the surface of synergistic interaction analysis to determine positive or negative interactions between drug combinations, such as morphine + dipyrone (López-Muñoz, 1994), morphine + aspirin (López-Muñoz et al., 1995), D-propoxyphene + aspirin (López-Muñoz, 1995), and D-propoxyphene + acetaminophen (López-Muñoz and Salazar, 1995), all of which were found to produce different degrees of antinociceptive potentiation. This analysis permits the evaluation and determination of analgesic drug doses that will exert maximal potentiating effects. It is therefore expected that this approach will have significant implications for the treatment of pain (López-Muñoz, 1994).

The purpose of analgesic drug combinations is to optimize dose regimens so that greater analgesic effects are obtained with decreased unwanted side effects. Then, administration of combinations of drugs like morphine with NSAIDs can lead to the use of lower doses of opioid drugs with increased therapeutic effects (Wei-wu et al., 1999; Picard et al., 1997). It has been demonstrated that the combination of morphine with some NSAIDs increases antinociceptive effects and decreases adverse events (Hernández-Delgadillo et al., 2002). The results obtained in this study showed a positive synergism between ketorolac and tramadol; over the dose ranges used, the antinociceptive activities of ketorolac and tramadol given individually tended to be less than those observed when they were administered in combination. Potentiation of the antinociceptive effects was noticed when a low dose of tramadol was used with all the doses of ketorolac, indicating that the synergistic interaction depends on the dose. Similar results were reported using either tramadol/flurbiprofen (Doroschak et al., 1999) or tramadol/acetaminophen (Medve et al., 2001; Mullican and Lacy, 2001). Doroschak et al. (1999) evaluated the combination of flurbiprofen and tramadol for management of endodontic pain, and their study indicated that the combination of an NSAID with tramadol provides superior pain relief, compared with either drug alone. In the same study, the incidence of adverse effects was recorded in patients, but the differences between groups were not significant. Similar results have been obtained in the present study, although the study design and experimental model were different. Picard et al. (1997) conducted a prospective randomised double-blind comparison of patient-controlled analgesia, and the results showed that the combination of morphine and ketorolac was more effective than morphine or ketorolac alone in relieving rest pain throughout the study. Combined therapy with ketorolac and analgesic opioid results in a 25–50% reduction in opioid requirements, and in some patients, this is accompanied by a concomitant decrease in opioid-induced adverse events, more rapid return to normal gastrointestinal function, and shorter stay in the hospital (Gillis and Brogden, 1997; Carney et al., 2001).

In order to find the advantages of combining ketorolac with tramadol, we searched for gastric injuries as a reflection of unwanted gastric side effects. The doses of ketorolac (1.0 mg/kg) plus tramadol (17.8 mg/kg) were selected for the gastric effect of the drugs because this combination produced the maximum antinociceptive effect. Our results showed that the adverse effects could be reduced; that is, the incidence of gastrointestinal adverse events (erosions) was lower with ketorolac + tramadol than with ketorolac or indomethacin alone, whereas ketorolac + tramadol was also able to generate ulcers (low percent); these adverse effects

were similar to that produced by ketorolac alone. This is important because the combination did not produce a higher incidence of side effects than that produced by each drug alone; instead, the results of synergistic antinociceptive interaction reflect a potentiation type of interaction. Further experiments will be required to determine the mechanisms involved in these effects. Previous studies have shown that the combination of morphine with some NSAIDs can activate the serotonergic (Sandrini et al., 1998) and the opioid (Maves et al., 1994) systems, and evidence has also been provided for the participation of the nitric oxide–cGMP pathway and other mechanisms such as activation of opioid and prostanoid receptors. It has been proposed that opioids produce analgesia within the midbrain periaqueductal grey by inhibiting gamma-aminobutyric acid (GABA) system on neurones, which form part of a descending antinociceptive pathway, and microinjections of cyclooxygenase inhibitors into the periaqueductal grey produce analgesia (Tortorici and Vanegas, 1995). Vaughan et al. (1997) have hypothesized a mechanism that involves opioid modulation of arachidonic acid metabolites in GABA interneurons. These authors demonstrated that opioids might be coupled to a voltage-dependent potassium conductance via a pathway involving phospholipase A_2, arachidonic acid, and 12-lipoxygenase. Cyclooxygenase inhibitors potentiate opioid inhibition of GABA synaptic transmission, presumably because more arachidonic acid is available for enzymatic conversion to 12-lipoxygenase products (Vaughan et al., 1997). Therefore, it was demonstrated that inhibition of cyclooxygenase-1, rather than of cyclooxygenase-2, potentiates the inhibitory action of opioids on GABA synaptic transmission (Vaughan, 1998). In our study, it was shown that ketorolac, which is a nonselective cyclooxygenase inhibitor, has an important interaction with tramadol. Although this mechanism is hypothetical, the GABA interneurons might be suggested as a suitable site for the opioid/NSAID synergistic interaction to take place. However, other pharmacodynamic/pharmacokinetic interactions cannot be excluded. In addition to possible pharmacodynamic mechanism of the combination antinociception, ketorolac may alter the pharmacokinetic properties of the tramadol. The authors are not aware of any data that answer this question; however, future studies will address this issue.

In summary, this study quantified the antinociceptive synergy between ketorolac and tramadol during arthritic nociception in the rat. From these data, we conclude that: (1) oral ketorolac is a powerful potentiator of tramadol antinociception during arthritic nociception in the rat; (2) oral coadministration of ketorolac and tramadol produced an antinociceptive effect greater than that observed after individual treatment; (3) the potentiated antinociceptive effects were not accompanied by increased side effects; (4) the fact that the combinations of ketorolac + tramadol produced many potentiation effects is interesting; and (5) the consumption of ketorolac or tramadol was significantly lower when the two drugs were administered together. In addition "to improving the analgesia we may provide to the patients, we may also decrease or avoid adverse drug effects by reducing the analgesic dose requirement" (Maves et al., 1994).

Acknowledgements

We wish to thank A. Huerta, L. Oliva, and F. Sánchez for technical assistance. M. Déciga Campos is a fellow of the National Council for Science and Technology (CONACYT), Mexico. Ketorolac tromethamine and tramadol hydrochloride were generously provided by "Lab. RIMSA de México" whom the authors wish to thank.

References

Carlsson, K.H., Monzel, W., Jurna, I., 1988. Depression by morphine and the non-opioid analgesic agents, metamizol (dipyrone), lysine acetylsalicylate and paracetamol, of activity in the rat thalamus neurons evoked by electrical stimulation of nociceptive afferents. Pain 32, 313–326.

Carney, D.E., Nicolette, L.A., Ratner, M.H., Minerd, A., Baesl, T.J., 2001. Ketorolac reduces postoperative narcotic requirements. J. Pediatr. Surg. 36, 76–79.

Catapano, M.S., 1996. The analgesic efficacy of ketorolac for acute pain. J. Emerg. Med. 14, 67–75.

Covino, B.G., Dubner, R., Gybels, J., Kosterlitz, H.W., Liebeskind, J.C., Sternbach, R.A., Vyclicky, L., Yamamura, H., Zimmermann, M., 1980. Ethical standards for investigations of experimental pain in animals. Pain 9, 141–143.

Déciga-Campos, M., Guevara, U., Díaz, M.I., López-Muñoz, F.J., 2003. Enhancement of antinociception by co-administration of an opioid drug (morphine) and a preferential cyclooxygenase-2 inhibitor (rofecoxib) in rats. Eur. J. Pharmacol. 460, 99–107.

Doroschak, A.M., Bowles, W.R., Hargreaves, K.M., 1999. Evaluation of the combination of flurbiprofen and tramadol for management of endodontic pain. J. Endod. 25, 660–663.

Duarte, I.D., Santos, I.R., Lorenzetti, B.B., Ferreira, S.H., 1992. Analgesia by direct antagonism of nociceptor sensitization involves the arginine–nitric oxide–cGMP pathway. Eur. J. Pharmacol. 217, 225–227.

Ferreira, S.H., Vane, J.R., 1974. New aspects of the mode of action of nonsteroid anti-inflammatory drugs. Annu. Rev. Pharmacol. 14, 57–73.

Forrest, J.B., Camu, F., Geer, I.A., Kehlet, H., Abdalla, M., Bonnet, F., Ebrahim, S., Escolar, G., Jage, J., Pococi, J., Velo, G., Langman, M., Bianchi, P.G., Samama, M.M., Hetlinger, E., 2002. Ketorolac, diclofenac, and ketoprofen are equally safe for pain relief after surgery. Br. J. Anaesth. 88, 227–233.

Gillis, J.C., Brogden, R.N., 1997. Ketorolac. A reappraisal of its pharmacodynamic and pharmacokinetic properties and therapeutic use in pain management. Drugs 53, 139–188.

Griffin, M.R., 1998. Epidemiology of nonsteroidal anti-inflammatory drug-associated gastrointestinal injury. Am. J. Med. 104 (3A), 23S–29S.

Hernández-Delgadillo, G.P., Ventura, R., Díaz, M.I., Domínguez, A.M., López-Muñoz, F.J., 2002. Metamizol potentiates morphine antinociception but not constipation after chronic treatment. Eur. J. Pharmacol. 441, 177–183.

Lee, Y.H., Mollison, K.W., Cheng, W.D., 1971. The effects of anti-ulcer agents on indomethacin-induced gastric ulceration in the rat. Arch. Int. Pharmacodyn. 191, 370–377.

Lewis, K.S., Han, N.H., 1997. Tramadol: a new centrally acting analgesic. Am. J. Health-Syst. Pharm. 54, 643–652.

López-Muñoz, F.J., 1994. Surface of synergistic interaction between dipyrone and morphine in the PIFIR model. Drug Dev. Res. 33, 26–32.

López-Muñoz, F.J., 1995. Profile of analgesic interaction between aspirin and D-propoxyphene obtained by means of the surface of synergistic interaction. Drug Dev. Res. 35 (1), 13–19.

López-Muñoz, F.J., Salazar, L.A., 1995. Determination of analgesic interaction between acetaminophen and D-propoxyphene obtained by means of the surface of synergistic interaction. Methods Find. Exp. Clin. Pharmacol. 17 (5), 311–320.

López-Muñoz, F.J., Castañeda-Hernández, G., Villalón, C.M., Terrón, J.A., Salazar, L.A., 1993a. Analgesic effects of combinations contain opioid drugs with either aspirin or acetaminophen in the rat. Drug Dev. Res. 29, 229–304.

López-Muñoz, F.J., Salazar, L.A., Castañeda-Hernández, G., Villarreal, J.F., 1993b. A new model to asses analgesic activity: pain-induced functional impairment in the rat (PIFIR). Drug Dev. Res. 28, 169–175.

López-Muñoz, F.J., Villalón, C.M., Terrón, J.A., Salazar, L.A., 1994. Analgesic interactions produced by combinations of dipyrone and morphine in the rat. Proc. West. Pharmacol. Soc. 37, 17–19.

López-Muñoz, F.J., Villalón, C.M., Terron, J.A., Salazar, L.A., 1995. Doses of acetyl salicylic acid and morphine in combination which provided either maximal levels of analgesia or the highest potentiation effect in the rat. Drug Dev. Res. 35 (2), 94–101.

Lorenzetti, B.B., Ferreira, S.H., 1985. Mode of analgesic action of dipyrone: direct antagonism of inflammatory hyperalgesia. Eur. J. Pharmacol. 114, 375–381.

Maves, T.J., Pechman, P.S., Meller, S.T., Gebhart, G.F., 1994. Ketorolac potentiates morphine antinociception during visceral nociception in the rat. Anesthesiology 80 (5), 1094–1101.

Medve, R.A., Wang, J., Karim, R., 2001. Tramadol and acetaminophen tablets for dental pain. Anesth. Prog. 48, 79–81.

Mullican, W.S., Lacy, J.R., 2001. Tramadol/acetaminophen combination tablets and codeine/acetaminophen combination capsules for the management of chronic pain: a comparative trial. Clin. Ther. 23, 1429–1445.

Picard, P., Bazin, J.E., Conio, N., Ruiz, F., Schoeffeler, P., 1997. Ketorolac potentiates morphine in postoperative patient-controlled analgesia. Pain 73, 401–406.

Rowland, M., Tozer, T.N., 1989. Clinical Pharmacokinetics: Concepts and Applications. 2nd ed. Lea and Febiger, Philadelphia, pp. 255–257, 459–463.

Salazar, L.A., Ventura-Martínez, R., López-Muñoz, F.J., 1995. Synergistic antinociceptive interaction between aspirin and tramadol, the atypical opioid analgesic, in the rat. Drug Dev. Res. 36, 119–124.

Sandrini, M., Ottani, A., Vitale, G., Pini, L.A., 1998. Acetylsalicylic acid potentiates the antinociceptive effect of morphine in the rat: involvement of the central serotonergic system. Eur. J. Pharmacol. 355 (2–3), 133–140.

Sandrini, M., Vitale, G., Ottani, A., Pini, L.A., 1999. The potentiation of analgesic activity of paracetamol plus morphine involves the serotonergic system in rat brain. Inflamm. Res. 48, 120–127.

Seegers, A.J.M., Jager, L.P., Zandberg, P., van Noordwijk, J., 1981. The antiinflammatory, analgesic and antipyretic activities of non-narcotic analgesic drug mixtures in rats. Arch. Int. Pharmacodyn. 251, 237–254.

Tallarida, R.J., Porreca, F., Cowan, A., 1989. Statistical analysis of drug–drug and site–site interactions with isobolograms. Life Sci. 45, 947–961.

Taylor, J., Mellstrom, B., Fernaud, I., Naranjo, J.R., 1998. Metamizol potentiates morphine effects on visceral pain and evoked c-Fos immunoreactivity in spinal cord. Eur. J. Pharmacol. 351, 39–47.

Tortorici, V., Vanegas, H., 1994. Putative role of medullary off- and on cells in the antinociception produced by dipyrone (metamizol) administered systematically or microinjected into PAG. Pain 57, 197–205.

Tortorici, V., Vanegas, H., 1995. Anti-nociception induced by systemic or PAG-microinjected lysine-acetylsalicylate in rats. Effects on tail-flick related activity of medullary off- and on-cells. Eur. J. Neurosci. 7, 1857–1865.

Vaughan, C.W., 1998. Enhancement of opioid inhibition of GABAergic synaptic transmission by cyclo-oxygenase inhibitors in rat periaqueductal grey neurons. Br. J. Pharmacol. 123, 1479–1481.

Vaughan, C.W., Ingram, S.L., Connor, M.A., Christie, M.J., 1997. How opioids inhibit GABA-mediated neurotransmission. Nature 390 (11), 611–614.

Wei-wu, P., Martin, S.M., Ming-Chou, K., Min, H.H., 1999. Patient-controlled analgesia with morphine plus lysine acetyl salicylate. Anesth. Analg. 89, 995–998.

Wideman, G.L., Keffer, M., Morris, E., Doyle, R.T., Jiang, J.G., Beaver, W.T., 1999. Analgesic efficacy of a combination of hydrocodone with ibuprofen in postoperative pain. Clin. Pharmacol. Ther. 65 (1), 66–76.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.