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Enhancement of antinociception by co-administration of an opioid drug (morphine) and a preferential cyclooxygenase-2 inhibitor (rofecoxib) in rats

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Abstract

Synergism has been used to obtain analgesia at doses at which side effects are minimal. In addition, it has been demonstrated that inhibition of cyclooxygenase-2 is responsible for the therapeutic effects of nonsteroidal anti-inflammatory drugs (NSAIDs). The aim of this study was to evaluate the antinociceptive interaction between the preferential COX-2 inhibitor, rofecoxib and morphine. Several combinations were evaluated using the pain-induced functional impairment model (PIFIR), a rat model of arthritic pain. Surface of synergistic interaction (SSI) analysis and an isobolographic method were used to detect the antinociceptive potency of the drugs, given either individually or in combination. 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. Male rats received orally morphine alone (10, 17.8, 31.6, 56.2 and 100.0 mg/kg), rofecoxib alone (3.2, 5.6, 10, 31.6, 56.2 and 74.0 mg/kg) or 12 different combinations of morphine and rofecoxib. Three combinations exhibited potentiation of antinociceptive effects (10 mg/kg of morphine with either 5.6, 10 or 31.6 mg/kg of rofecoxib), whereas the other nine combinations showed additive antinociceptive effects. The combination of morphine, 56.2 mg/kg (p.o.), and rofecoxib, 31.6 mg/kg (p.o.), produced the maximum antinociceptive effect ($P < 0.05$). This combination caused gastric injuries less severe than those observed with indomethacin, i.e. it reduced ulcers and erosion formation. The synergistic antinociceptive effects of rofecoxib and morphine are important and suggest that combinations with drugs may decrease the side effects associated with the use of nonselective NSAIDs. Furthermore, the present results suggest that combinations containing opioid drugs and selective COX-2 inhibitors may have clinical utility in pain therapy.

Keywords: Inflammatory pain; Cyclooxygenase-2; Morphine; Rofecoxib; Synergism

1. Introduction

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 maximized 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 prostaglandins, which sensitise nerve endings at the site of injury (Ferreira and Vane, 1974; Lorenzetti and Ferreira, 1985), and activation of nitric oxide synthetase in the injured tissue (Duarte et al., 1992; Granados-Soto et al., 1995). More recent evidence suggests that NSAID may also have direct

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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).

Data suggest that cyclooxygenase has two isoforms (Vane et al., 1998), a constitutive isoform cyclooxygenase-1, which is responsible, among other actions, for maintaining normal function in the gastrointestinal and renal tracts, and an inducible isoform cyclooxygenase-2, which is found in areas of inflammation and in the brain (Yamagata et al., 1993). It has been suggested that the anti-inflammatory actions of NSAIDs are due to inhibition of cyclooxygenase-2, whereas the unwanted side effects are due to inhibition of cyclooxygenase-1. The vast majority of NSAIDs currently available display no selectivity for cyclooxygenase-2 and can, thus, cause the adverse reactions so commonly seen during NSAID treatment (Langman et al., 1994). Several selective cyclooxygenase-2 inhibitors have been developed, and one of them is rofecoxib (MK-0966 or Vioxx: 4-[4-(methylsulfonyl)phenyl]-3-phenyl-2-(5*H*)-furanone). This compound displays antipyretic, anti-inflammatory and antinociceptive properties (Chan et al., 1999), and has no gastric toxicity and is well tolerated in humans (Day et al., 2000).

On the other hand, there are combinations of opioids and NSAIDs, which 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). The purpose of the present work was to investigate the antinociceptive effect of rofecoxib and morphine by administration alone or in combination, using the pain-induced functional impairment model (PIFIR model) in the rat, an animal model of arthritic pain (López-Muñoz et al., 1993b).

2. Materials and methods

2.1. Animals

Male 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; rofecoxib, Vioxx or 4-[4-(methylsulfonyl)-phenyl]-3-phenyl-2-(5*H*)-furanone) was obtained from Laboratories Menarini (Barcelona, Spain). Morphine hydrochloride (Mexican Secretariat of Health, Mexico City, Mexico), rofecoxib and indomethacin (Sigma) were dissolved in 0.5% carboxymethylcellulose. Rofecoxib, indomethacin and morphine 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 anaesthetized 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 (Beckton 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, rofecoxib and morphine 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 and 14:00.

2.4. Study design

The antinociceptive effects produced by rofecoxib and morphine given either individually or in combination were studied. First, each dose of rofecoxib (3.2, 5.6, 10, 31.6, 56.2 or 74.0 mg/kg) or morphine (10, 17.8, 31.6, 56.2 or 100.0 mg/kg) was given to six animals to obtain the corresponding dose–response curves (DRC), and the doses of rofecoxib (5.6, 10 or 31.6 mg/kg) and morphine (10, 17.8, 31.6 or 56.2 mg/kg) were then combined to analyse possible synergistic interactions (12 combinations in total). At the end of the experiment, the rats were euthanized.

2.5. Indomethacin-induced gastric erosions and ulcers

Male 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). Rofecoxib (52.2 mg/kg), morphine (31.6 mg/kg), vehicle (carboxymethylcellulose of 0.5%) and the combination of morphine and rofecoxib (31.6 and 52.2 mg/kg, respectively) were administered orally at the same time to five groups (six rats each); 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 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.

2.6. Data presentation and statistical evaluation

Data in the text, table and figures are expressed as the FI%. Curves for FI% versus 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 morphine and rofecoxib was calculated with surface of synergistic interaction (SSI) analysis (López-Muñoz et al., 1994) and an isobolographic method (Tallarida et al., 1989; Tallarida, 1992). 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 area under the curve (AUC) was obtained by the trapezoidal rule (Rowland and Tozer, 1989). All values for each treatment are means ± 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 morphine or rofecoxib (assayed separately) were compared with the AUC value obtained from the corresponding combination by analysis of variance (ANOVA) and Dunnett's test. $P < 0.05$ was considered statistically significant (*).

The isobologram was constructed using doses producing 50% of the maximum possible effect (ED_{50}) when the drugs were given alone or combined. To perform the isobolographic analysis, rofecoxib and morphine were administered in combination as fixed ratios of the equieffective ED_{50} dose for each drug (rofecoxib/morphine = 1:1). The ED_{50} values (± S.E.M.) for morphine and rofecoxib 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 rofecoxib and morphine.

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 for another 4 h. The rats that received vehicle (methylcellulose of 0.5%) did not show any significant recovery of the FI% during the observation period. At the doses used, morphine and rofecoxib 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).

3.2. Antinociceptive effects of drugs assayed individually

Fig. 1 shows the dose–response curves for morphine and rofecoxib. Both drugs increased AUC in a dose-dependent manner and displayed similar efficacy, i.e. they produced

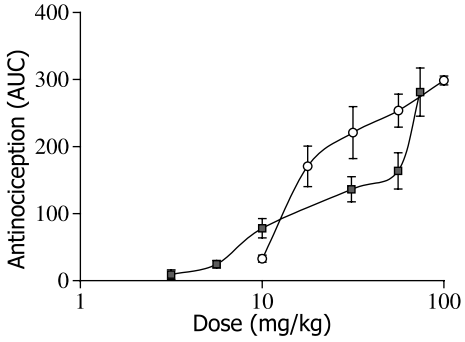

Fig. 1. Oral antinociceptive effect of morphine (○) and rofecoxib (■) in the pain-induced functional impairment model. The response is expressed on the *y*-axis as the AUC of the functionality index over the 4-h observation period (%·h). Data are expressed as means ± S.E.M. for six animals.

similar maximum effects. Thus, morphine showed a maximal antinociceptive effect of 298.5 ± 6.7 area units (au) and rofecoxib of 281.3 ± 35.9 area units. The ED_{50} values for the drugs indicate that there were significant differences in their antinociceptive potencies; morphine (ED_{50} = 22.2 mg/kg) was more potent than rofecoxib (ED_{50} = 69.2 mg/kg). There were no adverse effects with the doses used.

3.3. Antinociceptive effects of the drug combinations

Figs. 2–4 depict the antinociceptive effect from the 12 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 morphine + rofecoxib combinations (56.2 + 31.6 mg/kg, respectively, see Fig. 2) was 338.8 ± 9.4 area units. Statistical analysis of data from Fig. 2 indicates an interaction between rofecoxib and morphine ($P < 0.05$), 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, nine combinations of morphine and rofecoxib produced additive antinociceptive effects, and three produced potentiation with 95% confidence limits ($P < 0.05$) ([a] in Fig. 3). These combinations were 10 + 5.6, 10 + 10 and 10 + 31.6 mg/kg of morphine + rofecoxib, respectively.

In order to obtain the surface of synergistic interaction for the combinations morphine + rofecoxib, 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 morphine + rofecoxib, i.e. addition or potentiation. For example, three combinations of morphine + rofecoxib displayed potentiation and, interestingly, the dose of morphine was the same in all cases (see Table 1). Morphine, at a dose of 10 mg/kg, yielded an AUC of 32.7 ± 5.2 au and rofecoxib, at the dose of 5.6 mg/kg, rendered an AUC of 24.5 ± 5.7 au; however, the combination of morphine + rofecoxib (10 + 5.6 mg/kg) yielded an AUC of 120.1 ± 9.5 au, which is higher than the expected AUC resulting from the sum of the individual values, i.e. 57.2 au ($P < 0.05$). The analysis of the E_{max} from the

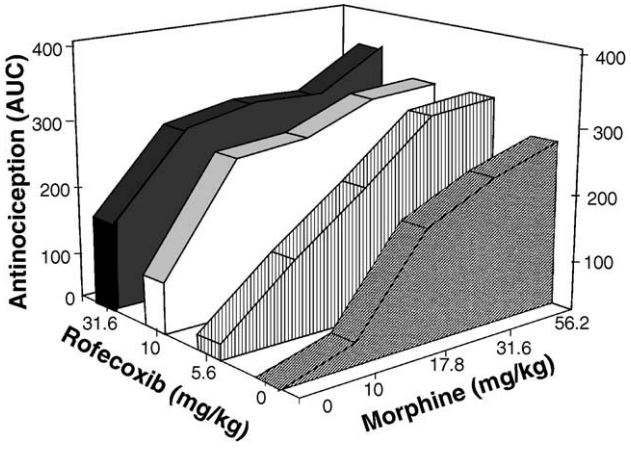

Fig. 2. Antinociceptive effects obtained with rofecoxib and morphine either alone or in combination. The *y*-axis represents the area under the curve (AUC) of the time course; the *x*-axis depicts the doses (mg/kg) of morphine administered simultaneously with rofecoxib; and the *z*-axis depicts the doses (mg/kg) of rofecoxib used to obtain the dose–response curves (DRC). The combination rofecoxib (31.6 mg/kg) + morphine (56.2 mg/kg) showed the greatest antinociceptive effect. Each point represents the mean of six experiments; there is an interaction between rofecoxib and morphine ($P < 0.05$).

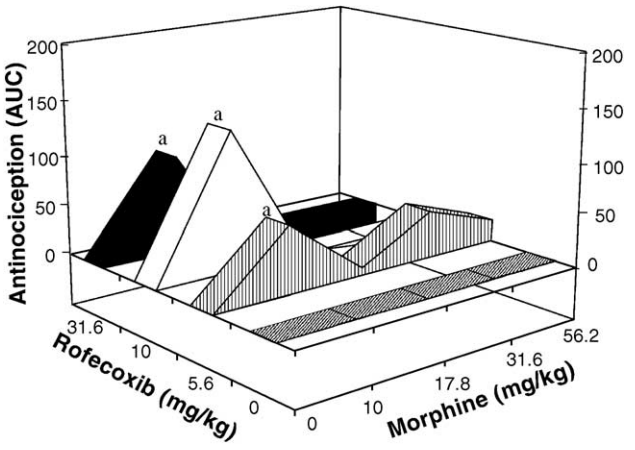

Fig. 3. The antinociceptive effects produced by the different combinations of rofecoxib and morphine after subtracting the individual effects. The axes are the same as those in Fig. 2. Three results correspond to potentiation of antinociceptive effects ($P < 0.05$), whereas the other nine combinations represent addition of antinociceptive effect. The combination of rofecoxib/morphine (10:10 mg/kg) produced higher potentiation. Each interaction is represented by the mean for six animals.

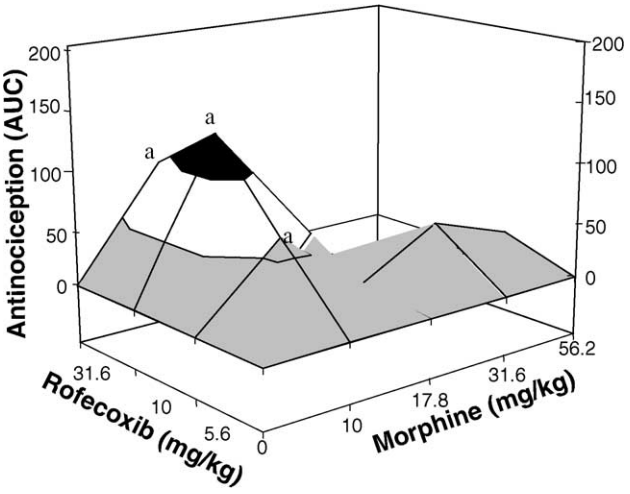

Fig. 4. Determination of the surface of synergistic interaction (SSI) for the combination rofecoxib + morphine. All the points of synergism from Fig. 3 have been joined by a plane. On this graph, the axes are the same as on Fig. 2. The doses producing either potentiation or addition, when co-administered, could easily be determined. Three combinations showed various degrees of potentiation, while others exhibited only additive antinociceptive effects.

corresponding time course curves showed an increase in the values obtained from the combination, which were higher than the corresponding values obtained from the arithmetic sum. Two other examples of potentiation with the same dose of morphine (10 mg/kg) and rofecoxib (10 and 31.6 mg/kg) are shown in Table 1.

The antinociceptive effects produced by the combinations that produced the maximum antinociceptive effect (31.6 mg/kg rofecoxib + 56.2 mg/kg morphine) and the combination that produced high potentiation (10.0 mg/kg rofecoxib + 10.0 mg/kg morphine) are shown in Fig. 5. As can be seen in Fig. 3A, the antinociception produced by

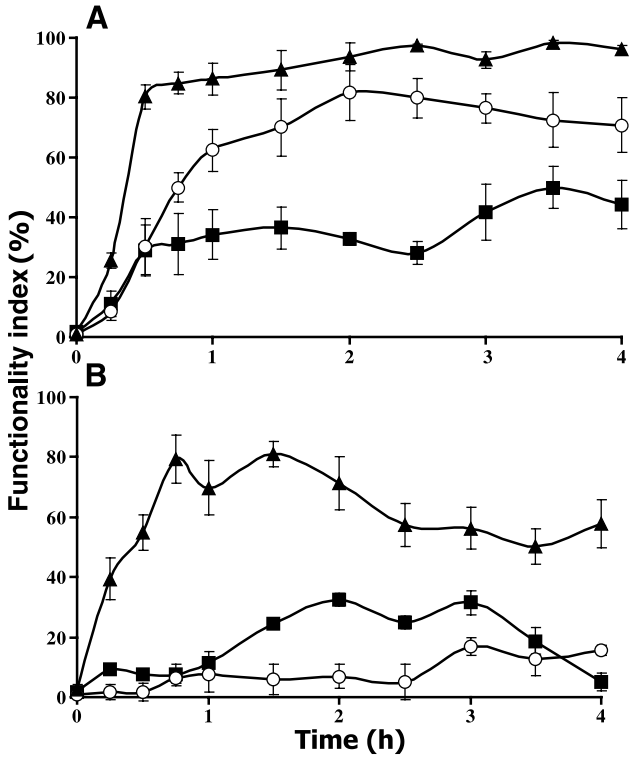

Fig. 5. Time courses of the combinations that produced (A) the maximum antinociceptive effect but not potentiation: 31.6 mg/kg rofecoxib (■), 56.2 mg/kg morphine (○) and the combination of morphine + rofecoxib (31.6 + 56.2 mg/kg) (▲); and (B) high potentiation: 10.0 mg/kg rofecoxib (■), 10.0 mg/kg morphine (○) and the combination of morphine + rofecoxib (10.0 + 10.0 mg/kg) (▲). This latter combination represents a clear example of potentiation of the antinociceptive effects; the AUC (242.3 ± 28.1 au) obtained with this combination was higher ($P < 0.01$) than the AUC obtained from the sum of the individual AUCs (78.1 ± 14.3 + 32.7 ± 5.2 au). Data are expressed as the means ± S.E.M. of six determinations.

Table 1
Three combinations with potentiation of antinociceptive effects of morphine + rofecoxib

Drug	Dose (mg/kg)	AUC (%·h) (x ± S.E.M.)[a]	E_{max} (FI%) (x ± S.E.M.)
Morphine	10	32.7 ± 5.2	16.9 ± 4.0
Rofecoxib	5.6	24.5 ± 5.7	12.4 ± 4.7
Morphine + rofecoxib	10 + 5.6	120.1 ± 9.5[b]	40.2 ± 11.9
Morphine	10	32.7 ± 5.2	16.9 ± 4.0
Rofecoxib	10	78.1 ± 14.3	32.6 ± 9.3
Morphine + rofecoxib	10 + 10	242.3 ± 28.1[b]	80.9 ± 4.2
Morphine	10	32.7 ± 5.2	16.9 ± 4.0
Rofecoxib	31.6	136.3 ± 18.7	36.4 ± 7.1
Morphine + rofecoxib	10 + 31.6	260.1 ± 31.7[b]	74.0 ± 9.4

[a] Area under the curve of the time course of the whole antinociceptive effect shown for the antinociceptive effect over 4 h, either alone or in combination.
[b] $P < 0.05$.

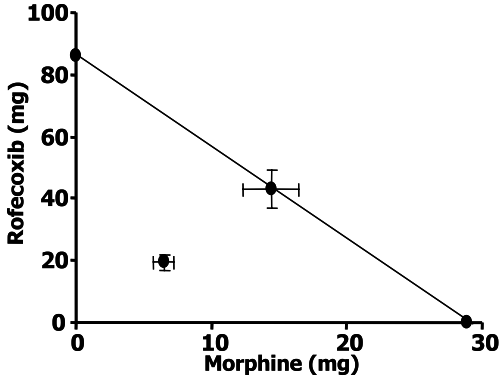

Fig. 6. Isobologram showing the antinociceptive interaction of rofecoxib and morphine in the PIFIR model. Horizontal and vertical bars indicate S.E.M. The oblique line between the x- and y-axes is the theoretical additive line. The point in the middle of this line is the theoretical additive point calculated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

rofecoxib + morphine (31.6 + 56.2 mg/kg) represented the maximum antinociceptive effect (which represents a total recovery) obtained with 338.8 ± 9.4 area units (au), while rofecoxib alone (31.6 mg/kg) showed an AUC of 136.2 ± 18.7 au and morphine alone (56.2 mg/kg) produced 253.6 ± 28.0 au only. This result was important if it is considered that the maximum morphine dose used (100.0 mg/kg) produced less antinociceptive effect: 298.5 ± 6.7 au. The combination depicted in Fig. 3B (10.0 mg/kg rofecoxib + 10.0 mg/kg morphine) merely represents a combination that produced the maximum potentiation of the antinociceptive effect (118.6% more AUC or whole antinociceptive effect than the sum of individual AUCs); likewise, both the time course and AUC (242.3 ± 28.1 au) obtained with this combination were higher ($P < 0.05$) than the respective values obtained with the sum of individual agents (110.8 au). The antinociception produced by rofecoxib + morphine (10.0 + 10.0 mg/kg) was 242.3 ± 28.1 au, while rofecoxib alone (10.0 mg/kg) showed an AUC of 78.1 ± 14.3 au and morphine alone (10.0 mg/kg) produced 32.7 ± 5.2 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; Tallarida, 1992). An isobologram showing the antinociceptive interaction of rofecoxib and morphine 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 ($P < 0.05$).

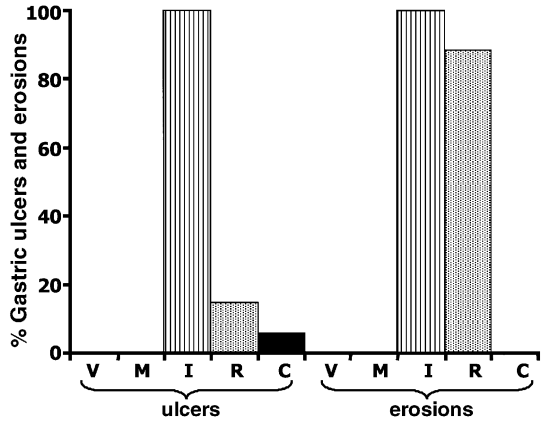

Fig. 7. Percent of gastric ulcers and erosions produced after two oral administrations of morphine (31.6 mg/kg; M), rofecoxib (56.2 mg/kg; R) or the combination morphine + rofecoxib (31.6 + 56.2 mg/kg; C). Indomethacin (20 mg/kg; I) and vehicle (carboxymethylcellulose of 5%; V) were taken as positive and negative controls, which represent the 100% and 0%, respectively. Each bar represents the mean for six animals.

3.4. Indomethacin-induced gastric ulcers and erosion

The administration of morphine did not produce ulcers or erosions. Its adverse effects were similar to those of vehicle. However, rofecoxib generated a lower percent of ulcers (14.7%) and less severe erosions (88.7%) than did indomethacin, 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%). Interestingly, the combination morphine + rofecoxib decreased the harshness of ulcerations and blocked the generation of erosions (Fig. 7).

4. Discussion

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 morphine or rofecoxib alone were selected on an increasing 0.25 logarithmic unit basis. The doses used for analysing the combinations (surface of synergistic interaction analysis) were selected from the respective dose–response curves. The selected doses of both rofecoxib and morphine are apparently high; at the dose of 10 mg/kg, rofecoxib completely inhibits carrageenan-induced hyperalgesia in rats (Chan et al., 1999); at the same dose, rofecoxib exhibits an anti-inflammatory action (Wallace et al., 1999; Wallace, 2001). However, these values (doses of morphine or rofecoxib used in our study) may result from the use of another nociceptive test, the PIFIR model.

Few reports have been published on analgesic combinations and few have demonstrated antinociceptive potentiation such as the case of morphine with some NSAIDs (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). We now examined the antinociceptive effects of morphine and rofecoxib both on single administration and in combination. The results show that the combination of morphine and rofecoxib does indeed display antinociceptive potentiation.

The NSAIDs act by inhibiting the activity of cyclooxygenase (Vane, 1971). Indeed, systemic administration of acetaminophen, aspirin (López-Muñoz et al., 1993a) or dipyrone (López-Muñoz, 1994), as well as intracerebroventricular administration of (*S*)-ketoprofen (López-Muñoz et al., 1998), produced clear antinociceptive effects in the PIFIR model. Thus, using nonselective cyclooxygenase inhibitors, it was demonstrated that both peripheral and central prostaglandins are involved in this model of nociception (Ventura et al., 2000). There are two isoforms of cyclooxygenase: a widely distributed isoform that is constitutively expressed, cyclooxygenase-1, and an inducible isoform that is prominent in inflammation sites, cycloox-

ygenase-2 (Jouzeau et al., 1997; Vane et al., 1998). Highly selective inhibitors of cyclooxygenase-2 have recently been developed that demonstrate anti-inflammatory and analgesic activity similar to that observed with NSAIDs (Jackson and Hawkey, 2000). One of these drugs is rofecoxib, which is a selective and potent inhibitor of cyclooxygenase-2 (Ehrich et al., 1999; Chan et al., 1999). Concentrations required to inhibit cyclooxygenase-2 activity by 50% (IC_{50}) were 0.018–0.046 μmol /l (Ehrich et al., 1999; Chan et al., 1999), which contrast with those required to inhibit cyclooxygenase-1, i.e. >50 μmol/l (Chan et al., 1999). Rofecoxib has approximately 1000-fold greater selectivity for cyclooxygenase-2 than for cyclooxygenase-1 (Chan et al., 1999). Our results showed that rofecoxib produced a dose-dependent antinociceptive effect after systemic administration. The data now obtained, in addition to those reported by Ventura et al. (2000) that demonstrate a local contribution of cyclooxygenase-2 in the PIFIR model, are in agreement with the idea that prostaglandins synthesised by cyclooxygenase-2 have an important role in the nociceptive process triggered by uric acid in the knee joint.

This is the first study analysing the effects of combinations of morphine and rofecoxib. One of our goals was to identify possible interactions between these drugs, as some combinations with opioids and NSAIDs have been shown to generate either additive or potentiated antinociceptive effects. On the other hand, there are no reports on the effect of combinations of morphine with selective cyclooxygenase-2 inhibitors. We analysed twelve combinations and observed that nine of these produced addition while the remaining three combinations produced potentiation. These results were analysed by surface of synergistic interaction since our group has used this 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 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 results obtained in this study showed a positive synergism between morphine and rofecoxib; over the dose ranges used, the antinociceptive activities of rofecoxib and morphine 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 morphine was used with all the doses of rofecoxib, indicating that the synergistic interaction depends on the dose. This was the major reason to search for antinociceptive interactions using a range of combinations since it allows selection of the most effective combination.

The surface of synergistic interaction analysis was applied with different ratios of the combination of morphine with rofecoxib, which allowed us to determine the maximum antinociceptive effect with a group of combinations. Our findings showed that the combination of morphine with rofecoxib is able to produce potentiated antinociceptive effects. It is interesting to note that the time to onset of the antinociceptive effect produced by the combinations was shorter than that observed with morphine or rofecoxib alone. It is, thus, clear that morphine can generate potentiated antinociceptive effects when given with both nonselective (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) and selective (present results) inhibitors of COX. The potentiation effects (seen when morphine + rofecoxib were administered) were confirmed with an isobolographic method.

The purpose of analgesic drug combinations is to optimize dose regimens so that greater analgesic effects are obtained with decreased unwanted side effects. The most common adverse effects in patients on chronic morphine treatment are respiratory depression, constipation, nausea, vomiting, lightheadedness, dizziness, sedation, dysphoria, euphoria and sweating (Cherny, 1996). Then, administration of combinations of morphine with NSAIDs will lead to the use of lower doses of morphine 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; in order to find the advantages of combining morphine with rofecoxib, we searched for gastric injuries as a reflection of unwanted gastric side effects. The doses of morphine (56.2 mg/kg) plus rofecoxib (31.6 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, i.e. the incidence of gastrointestinal adverse events was lower with rofecoxib than with indomethacin though rofecoxib was also able to generate ulcers (low percent) and erosions and these adverse effects were decreased when the drug was given in combination with morphine. The erosions can evolve to ulcers (Brzozowski et al., 1999), thus, rofecoxib might generate gastric injury under chronic treatment. This is important because there is no evidence in the literature to support the occurrence of gastrointestinal side effects for rofecoxib in naive animals (Laudanno et al., 2001). In contrast, some studies indicate that the selective cyclooxygenase-2 inhibitors, including rofecoxib, raise concern regarding the risk of causing cardiovascular toxicity by disrupting the balance that exists between the production of prostacyclin and thromboxane (Bombardier et al., 2000). Our data are in agreement with clinical observations indicating that the incidence of gastrointestinal adverse events (perforations, ulcerations and bleeding) was lower for rofecoxib than for traditional NSAIDs (Bombardier et al., 2000; Matheson and Figgitt, 2001). Interestingly, the combination of morphine with rofecoxib not only decreased the level of ulcers but also the erosions. Further experiments will be

required to determine the mechanisms involved in these effects.

The mechanism of the synergistic interactions between morphine and rofecoxib is unknown. 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 NO-cyclic GMP pathway (Aguirre-Bañuelos and Granados-Soto, 1999) 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 system on neurons which form part of a descending antinociceptive pathway and microinjections of cyclooxygenase inhibitors into the periaqueductal grey, thus, producing analgesia (Tortorici and Vanegas, 1995). Vaughan et al. (1997) have hypothesized a mechanism that involves opioid modulation of arachidonic acid metabolites in gamma-aminobutyric acid 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 gamma-aminobutyric acid 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 gamma-aminobutyric acid synaptic transmission (Vaughan, 1998). In our study, however, it was shown that rofecoxib, which is a cyclooxygenase-2 inhibitor, has an important interaction with morphine. This contradiction may be explained by the fact that the Vaughan proposal was based on in vitro work and our study was in vivo. 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 summary, (1) systemic co-administration of morphine and rofecoxib produced an antinociceptive effect greater than that observed after individual treatment; (2) the potentiated antinociceptive effects were not accompanied by increased side effects; and (3) the fact that the combination of morphine and rofecoxib eliminates the gastric erosions produced by rofecoxib is interesting. These results have potential therapeutic usefulness in the treatment of pain.

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References

Aguirre-Bañuelos, P., Granados-Soto, V., 1999. Evidence for a peripheral mechanism of action for the potentiation of the antinociceptive effect of morphine by dipyrone. J. Pharmacol. Toxicol. 42, 79–85.

Bombardier, C., Laine, L., Reicin, A., Shapiro, D., Burgos-Vargas, R., Davis, B., Day, R., Ferraz, M.B., Hawkey, C.J., Hochberg, M.C., Kvien, T.K., Schnitzer, T.J., 2000. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. New Engl. J. Med. 343, 1520–1528.

Brzozowski, T., Konturek, P.C., Konturek, S.J., Silwowski, Z., Drozdowicz, D., Stachura, J., Pajdo, R., Hahn, E.G., 1999. Role of prostaglandins generated by cyclooxygenase-1 and cyclooxygenase-2 in healing of ischemia–reperfusion-induced gastric lesions. Eur. J. Pharmacol. 385, 47–61.

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.

Chan, C.C., Boyce, S., Brideau, C., Charleson, S., Cromlish, W., Ethier, D., Evans, J., Ford-Hutchinson, A.W., Forrest, M.J., Gauthier, J.Y., Gordon, R., Gresser, M., Guay, J., Kargman, S., Kennedy, B., Leblanc, Y., Leger, S., Mancini, J., O'Neill, G.P., Oullet, M., Patrick, D., Percival, D., Perrier, H., Prasit, P., Rodger, I., Tagari, P., Therien, M., Vickers, P., Visco, D., Wang, Z., Webb, J., Wong, E., Xu, J.L., Young, R.N., Zamboni, R., Riendeau, D., 1999. Rofecoxib [Vioxx; MK-0966; 4-[4′-methylsulfonylphenyl]3-phenyl-2-(5*H*)-furanone]; a potent and orally active cyclooxygenase-2-inhibitor. Pharmacological and biochemical profiles. J. Pharmacol. Exp. Ther. 290, 551–560.

Cherny, N.I., 1996. Opioid analgesics. Comparative features and prescribing guidelines. Drugs 51 (5), 713–737.

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.

Day, R., Morrison, B., Luza, A., Castañeda, O., Strusberg, A., Nahir, M., Helgetveit, B., Kress, B., Daniels, B., Bolongnese, J., Krupa, D., Seidenberg, B., Ehrich, E., 2000. A randomized trial of the efficacy and tolerability of the COX-2 inhibitors rofecoxib vs. ibuprofen in patients with osteoarthritis. Rofecoxib/ibuprofen comparator study group. Arch. Intern. Med. 26–160 (12), 1781–1787.

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.

Ehrich, E.W., Dallob, A., De Lepeleire, I., 1999. Characterization of rofecoxib as a cyclooxygenase-2 isoform inhibitor and demonstration of analgesia in the dental pain model. Clin. Pharmacol. Ther. 65, 336–347.

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.

Granados-Soto, V., Flores-Murrieta, F., Castañeda-Hernández, G., López-Muñoz, F.J., 1995. Evidence for the involvement of nitric oxide in the antinociceptive effect of ketorolac. Eur. J. Pharmacol. 277, 281–284.

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

Jackson, L.M., Hawkey, C.J., 2000. COX-2 selective nonsteroidal anti-inflammatory drugs. Do they really offer any advantages? Drugs 59 (6), 1207–1216.

Jouzeau, J.Y., Terlain, B., Abid, A., Nédélec, E., Netter, P., 1997. Cyclooxygenase isoenzymes. How recent findings affect thinking about nonsteroidal anti-inflammatory drugs. Drugs 53 (4), 563–582.

Langman, J.S., Weil, J., Wainwright, P., 1994. Risks of bleeding peptic ulcer associated with individual non-steroidal anti-inflammatory drugs. Lancet 343, 1075–1078.

Laudanno, O.M., Cesolari, J.A., Esnarriaga, J., Rista, L., Piombo, G., Maglione, C., Aramberry, L., Sambrano, J., Godoy, A., Rocaspana,

A., 2001. Gastrointestinal damage induced by celecoxib and rofecoxib in rats. Dig. Dis. Sci. 46 (4), 779–784.

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.

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., Villareal, 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., Terron, 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.

López-Muñoz, F.J., Ventura, M.R., Díaz, R.I., Fernández-Guasti, A., Tost, D., Cabré, F., Mauleón, D., 1998. Antinociceptive effects of *S*(+)-ketoprofen and other analgesic drugs in a rat model of pain induced by uric acid. J. Clin. Pharmacol. 38, 11S–21S.

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

Matheson, A.J., Figgitt, D.P., 2001. Rofecoxib. A review of its use in the management of osteoarthritis, acute pain and rheumatoid arthritis. Drugs 61 (6), 833–855.

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.

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 & Febiger, Philadelphia, London, 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., 1992. Statistical analysis of drug combinations for synergism. Pain 49, 93–97.

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.

Vane, J.R., 1971. Inhibition of prostaglandin synthesis as a mechanism of action for the aspirin-like drugs. Nature 231, 232–235.

Vane, J.R., Bakhle, Y.S., Botting, R.M., 1998. Cyclooxygenases 1 and 2. Annu. Rev. Pharmacol. Toxicol. 38, 97–120.

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.

Ventura, M.R., Díaz, R.I., Hernández, G.P., Domínguez, R.A., Salazar, L.A., Cabré, F., López-Muñoz, F.J., 2000. Role of COX-1 and COX-2 synthesized prostaglandins in a rat model of arthritic pain. Drug Dev. Res. 51, 253–259.

Wallace, J.L., 2001. Mechanisms of protection and healing: current knowledge and future research. Am. J. Med. 110, 19S–23S.

Wallace, J.L., Chapman, K., McKnight, W., 1999. Limited anti-inflammatory efficacy of cyclo-oxygenase-2 inhibition in carrageenan-airpouch inflammation. Br. J. Pharmacol. 126, 1200–1204.

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.

Yamagata, K., Andreasson, K.I., Kaufman, W.E., Barnes, C.A., Worley, P.F., 1993. Expression of a mitogen-inducible cyclooxygenase in brain neurons; regulation by synaptic activity and glucocorticoids. Neuron 11, 371–386.

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