Pharmacology, Biochemistry and Behavior 91 (2008) 196–201

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

The antinociceptive efficacy of morphine, metamizol, or their combination in an experimental rat model with different levels of inflammatory pain

Francisco Javier López-Muñoz [a,d,*], Beatriz Godínez-Chaparro [a], Juan Carlos Huerta-Cruz [a], Uriah Guevara-López [b,c], Adriana Miriam Domínguez-Ramírez [d], Alma Rosa Cortés-Arroyo [d]

[a] Departamento de Farmacobiología, Cinvestav-Sede Sur, México D.F., México
[b] Unidad de Medicina del Dolor y Paliativa, Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán", México, D.F., México
[c] Investigador de la Unidad Médica de Alta Especialidad "Magdalena de las Salinas", Instituto Mexicano del Seguro Social, México, D.F., México
[d] Departamento de Sistemas Biológicos, Universidad Autónoma Metropolitana-Xochimilco, México, D.F., México

ARTICLE INFO

Article history:
Received 4 April 2008
Received in revised form 24 June 2008
Accepted 9 July 2008
Available online 20 July 2008

Keywords:
Nociception
Pain
Synergism
Morphine
Metamizol
PIFIR model

ABSTRACT

The purpose of this work was to evaluate the antinociceptive efficacy of an optimal morphine and metamizol combination on different levels of nociception (levels I, II, and III) using the "Pain-induced functional impairment model in the rat". The effect of acetylsalicylic acid was examined as a reference drug at the same levels of nociception. The antinociceptive effects produced by morphine (3.2 mg/kg s.c.) and metamizol (177.8 mg/kg s.c.) were studied either individually or in combination. The antinociceptive efficacies were expressed as either areas under the curve (AUCs), maximum effects as functionality index in percent of the time course, or the antinociceptive effects produced at 2 h after administration. Unlike morphine, the antinociceptive effects of acetylsalicylic acid decreased with increasing intensity of nociception. In summary, the analysis of antinociceptive efficacies produced by the co-administration of these drugs for different levels of nociception revealed that co-administration provided potentiated and better antinociceptive coverage throughout our observation time than did the individual drugs or the expected theoretical sum (using AUC or effects after 2 h). This is the first study to demonstrate that an optimal morphine and metamizol combination is able to produce potentiation of antinociceptive effects during intense pain.

1. Introduction

Morphine is the drug of choice for the treatment of moderate to severe pain (Martin and Eisenach, 2001). Its effects through μ-opioid receptors produce analgesia, mood effects, and rewarding behavior, and these effects also alter respiratory, cardiovascular, gastrointestinal, and neuroendocrine functions (Gutstein and Akil, 2006). Metamizol, the pirazolone derivative also known as dipyrone, is a nonsteroidal anti-inflammatory drug (NSAID) that acts as an effective analgesic and antipyretic agent. Additional beneficial effects of metamizol, such as its actions as a vascular smooth muscle relaxant (Hertle and Nawrath, 1984), antiapoptotic agent (Pompeia et al., 2001), and anticonvulsant (Reis et al., 2003), have been described. It has been banned in the USA and Sweden because of the potential side effect agranulocytosis, but it is widely used in Latin America, Germany, and other European countries for pain management due to its high efficacy and good gastric tolerability (García-Alonso et al., 1991; Sanchez et al., 2002). Metamizol and its active metabolites (4-methylaminoantipyrine and 4-aminoantipirine) may exert effect on inflammatory pain through the inhibition of prostaglandin synthesis in both the peripheral and the central nervous systems through inhibition of cyclooxygenase-2 activity. Moreover, Chandrasekharan et al. (2002) demonstrated that metamizol can decrease prostaglandin synthesis through the activation of cyclooxygenase-3. Other mechanisms of action, such as the activation of the NO-cGMP pathway in the periphery (Duarte et al., 1992), have been suggested to explain the antinociceptive effect of this drug. The central effects of metamizol have been associated with the activation of the endogenous opioid system (Tortorici et al., 1996), and a direct interaction of metamizol with the binding of glutamate on its receptors might partially explain its antinociceptive action (Beirith et al., 1998).

Combinations of analgesic drugs with different mechanisms of action may produce efficient analgesia and decreased side effects due to a reduction in the dosages for one or both compounds (Raffa, 2001; López-Muñoz et al., 2004). It is also important to notice that some combinations of analgesic drugs may not have clinical utility in pain therapy because combinatorial treatment can produce a sub-additive interaction (García-Hernández et al., 2007). One strategy for reducing the unwanted side effects of high doses of opioid analgesic drugs involves combining low doses of opioids with nonsteroidal anti-

* Corresponding author. Lab. No. 7 "Dolor y Analgesia" del Departamento de Farmacobiología, Cinvestav-Sede Sur, Calz. de los Tenorios No. 235 Col. Granjas Coapa, Deleg. Tlálpan, México D.F., C.P. 14330, México. Tel.: +52 55 54832851; fax: +52 55 54832863.
E-mail addresses: flopez@cinvestav.mx, flopezm@prodigy.net.mx (F.J. López-Muñoz).

0091-3057/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2008.07.007

inflammatory drug (López-Muñoz, 1994; Ripamonti and Dickenson, 2001; MacPherson, 2002). Preclinical studies have shown that metamizol increases morphine-induced antinociception when these drugs are co-administered, producing synergistic effects (López-Muñoz, 1994; López-Muñoz et al., 1994). The antinociceptive effects of these analgesic drugs used in combination as well as efficiency testing with a wide range of doses (metamizol 56.2 to 562.3 mg/kg s.c. and morphine 1 to 17.8 mg/kg s.c.) have been characterized in the "Pain-induced functional impairment model in the rat" (PIFIR model: López-Muñoz et al., 1993; López-Muñoz, 1994). Of the 24 combinations tested (PIFIR model using 30% uric acid intra-articular), 13 produced additive effects, and 11 showed supra-additive effects. The combination that resulted in the maximal antinociceptive potentiation was composed of 3.2 mg/kg morphine and 177.8 mg/kg metamizol. The purpose of this work was to evaluate and compare the antinociceptive effects of the morphine (3.2 mg/kg s.c.) and metamizol (177.8 mg/kg s.c.) combination that produced the maximal antinociceptive effect against moderate pain in a rat model at three different levels of nociception using the PIFIR model. In addition, a dose–response curve (DRC) for acetylsalicylic acid, an NSAID prototype, was determined at three different levels of nociception using the PIFIR model.

2. Materials and methods

2.1. Animals

Male Wistar rats [Crl:(WI)BR] (weight: 180 to 220 g) from UPEAL (UAM-X) were housed in an animal room at 22±2 °C with a 12:12 h light–dark cycle (lights on at 07:00) and free access to food and water. Experiments were performed during the light phase of the cycle. 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 Investigation of Experimental Pain in Animals (Zimmermann, 1983) and were approved by the local Committee on Ethics on Animal Experimentation. The number of experimental animals was kept to a minimum, and animals were used only once. At the end of the study, rats were euthanized in CO_2 to avoid unnecessary suffering.

2.2. Drugs

Acetylsalicylic acid was obtained from Bayer (Mexico City, Mexico); morphine hydrochloride was obtained from Mexican Secretariat of Health (Mexico City, Mexico); metamizol sodium was obtained from Sanofi-Aventis (Mexico City, Mexico). Acetylsalicylic acid was suspended in 0.5% carboxymethyl cellulose and administered orally; either morphine or metamizol was dissolved in isotonic saline solution and administered subcutaneously. Uric acid was obtained from Sigma Chemical Co and suspended in mineral oil. All drug solutions were freshly prepared and administered to the PIFIR model at volume of 2 ml/kg body weight for morphine and metamizol and 4 ml/kg body weight for acetylsalicylic acid. The doses mentioned in the text refer to salts of these substances.

2.3. Measurement of antinociceptive activity

Antinociceptive activity was assessed using the PIFIR model (López-Muñoz et al., 1993). Detailed methodology has been previously described. Briefly, the animals were anaesthetized with ether in an anesthesia chamber (Pyrex glass dryer saturated with ether vapor). To establish a level of moderate nociception (level I), an intra-articular (i.a.) injection of 0.05 ml of 30% uric acid suspended in mineral oil was administered to 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 with 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, 5 mm needle. To establish a level of sub-intense nociception (level II), we performed an i.a. injection of 0.05 ml of 50% uric acid suspended in mineral oil in the knee joint of the right hind limb. To establish the level of intense nociception (level III), we performed an i.a. injection of 50% uric acid in the knee joint of the right hind limb twice at an interval of one week. Immediately after the uric acid injection, an electrode was attached to the plantar surface of each hind paw (right and left) between the plantar pads. The rats were allowed to recover from anesthesia before being placed on a stainless steel cylinder of 30 cm diameter, which was rotated at 4 rpm to force the rats to walk for periods of 2 min every 30 min. Training periods were not necessary because the rats learned in the first minutes of the task. The time of contact between each electrode on the limbs of the rat and the cylinder was recorded with a computer. 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 injection with 30% uric acid, 2.0 h after injection with 50% uric acid, and 1.5 h after the second injection with 50% uric acid. At this time, the analgesic drugs were administered either alone or in combination. This time was considered as time zero for the measurement of the antinociceptive effect, which was measured every 30 min for the next 4 h. This methodology permitted determination of the time course of antinociceptive effects in the same animal. The data are expressed as the functionality index percent (FI%, the time of contact of the injected foot divided by the time of contact of the control left foot multiplied by 100). The following three pharmacological parameters of antinociceptive efficacy were selected and determined in the temporal course for analysis of the three different experimental conditions (levels of nociception): 1) the antinociceptive efficacies expressed as the area under the curve (AUC), 2) the maximum effect of each in time course, and 3) the antinociceptive effects produced at 2 h after administration. 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 experimental protocol consisted of two sets of experimental groups. In the first set, 12 groups of animals were treated with acetylsalicylic acid (56.2 to 1000 mg/kg p.o.) to obtain the corresponding DRC. In the second set, 9 groups of animals were treated with either morphine 3.2 mg/kg s.c., metamizol 177.8 mg/kg s.c., or the morphine and metamizol combination at the same doses. Nine groups were pre-administered 30% uric acid (level I), and the antinociceptive effects of either acetylsalicylic acid (56.2 to 1000 mg/kg), morphine (3.2 mg/kg), metamizol (177.8 mg/kg), or the morphine and metamizol combination were determined. The other 7 groups were pre-administered 50% uric acid (level II), and then the antinociceptive effect of either acetylsalicylic acid (177.8 to 1000 mg/kg), morphine, metamizol, or the combination was evaluated. Finally, the last five groups of rats were pre-administered 50% uric acid on two sequential weeks, and then the antinociceptive effects of either acetylsalicylic acid (562.3 or 1000 mg/kg), morphine, metamizol, or the combination was evaluated. Adequate controls were performed with each group using vehicles: one group of rats received an i.a. injection of uric acid; another group received an i.a. injection of mineral oil (vehicle of uric acid); another group received either isotonic saline solution s.c. (vehicle of morphine/metamizol) or carboxymethyl cellulose p.o. (vehicle of acetylsalicylic acid); the last group received an i.a. injection of uric acid and then (1:5, 2:0 or 2:5 h) either saline solution s.c. or carboxymethyl cellulose p.o.

2.5. Data analysis and statistics

Data in the text and figures are expressed as the FI%. Curves for FI% vs time were constructed for each treatment, and the corresponding time course was obtained. The cumulative antinociceptive effect during the whole observation period (4 h) was determined as the AUC in area units (au) of the time course to obtain the DRC (acetylsalicylic acid) or bar graphs. We analyzed the whole antinociceptive effect elicited by the analgesic agents either alone or in combination. The AUC was calculated for each drug combination and its 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 significantly 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 presented as mean±SEM for six animals. The AUC and FI% data obtained from the different treatments under three nociception levels were analyzed with two-way analyses of variance. Because of the significant interaction, we performed a one-way ANOVA using treatment×nociception level as a factor with post hoc multiple comparisons. When variances were homogeneous (Levene statistic), a Tukey test was used; otherwise, we applied a Dunnett T3 test. The FI% and AUC values for drug combinations were compared with the expected values using a one-tailed unpaired Student *t*-test with Bonferroni correction. SPSS software version 13 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses except one-tailed unpaired Student *t*-tests, which were performed using Microsoft Excel 2003 software (Microsoft, Redmond, WA, USA). Theoretical sum values, means, and standard deviations were obtained by summing the individual responses to the drugs and obtaining the square-root of the sum of the respective variances. *P*-values<0.05 were considered statistically significant.

3. Results

3.1. Nociceptive effect produced by uric acid

Intra-articular injection of mineral oil (vehicle of uric acid), subcutaneous injection of saline solution (vehicle of morphine or metamizol), and carboxymethyl cellulose p.o. (vehicle of acetylsalicylic acid) in rats without uric acid did not induce changes in the FI% during 6 h (Fig. 1). Intra-articular injection of uric acid induced a progressive dysfunction of the injured limb in the experimental conditions. When the FI% was zero, a persistent dysfunction of the injured limb lasted at least for 5 h. The analgesic drugs were evaluated only during the period of total dysfunction.

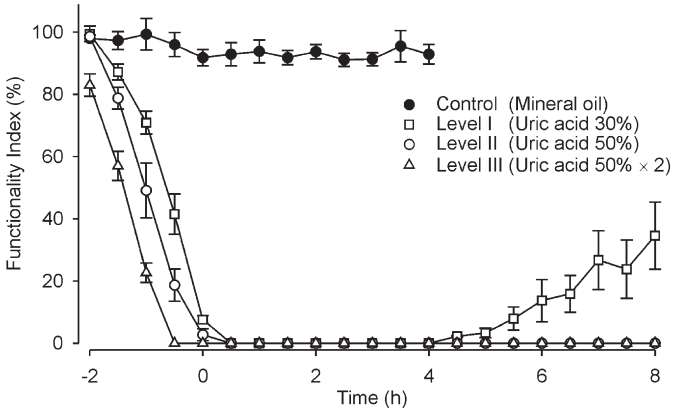

Fig. 1. Different levels of nociception in the pain-induced functional impairment model in the rat. The *y*-axis depicts the functionality index in %, and the *x*-axis shows the time in hours. Data are expressed as mean±SEM of six determinations. Intra-articular injection of uric acid induced a progressive dysfunction of the injured limb that reached its maximum 2.5 h after injection at nociception level I (□), 2.0 h after injection at nociception level II (○), and 1.5 h after injection at nociception level III (△).

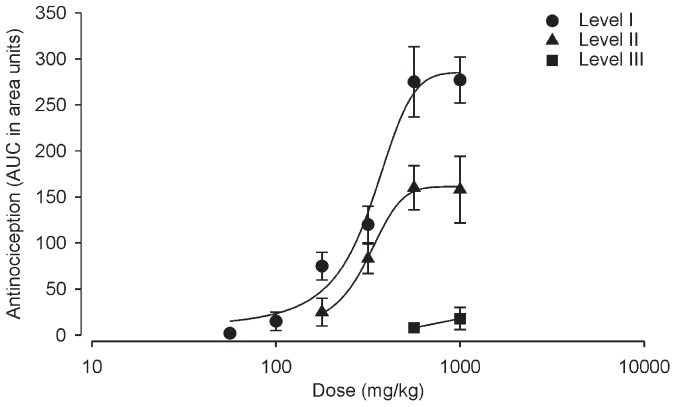

Fig. 2. Dose–response curves of acetylsalicylic acid after p.o. administration at three different levels of nociception. The *y*-axis depicts the antinociceptive effect of the drug, expressed as the AUC of the time course, over the 4 h observation period. The *x*-axis shows the dose of the drug in mg/kg. Data are expressed as mean±SEM of six determinations.

3.2. Antinociceptive activity of acetylsalicylic acid at different level of nociception

Fig. 2 shows the DRCs of acetylsalicylic acid after p.o. administration using the three different levels of nociception. The effect of acetylsalicylic acid was dose-dependent only at nociception levels I and II. The maximal effect was 275.3±38.1 au produced by 562.3 mg/kg on nociception level I, but the same dose produced a maximal effect of 160.4±24.3 au on nociception level II. A dose of 1000 mg/kg had a maximal effect of 18.3±12.0 au on nociception level III.

3.3. Antinociceptive activity of either morphine, metamizol, or the combination on different level of nociception

In Fig. 3, the antinociceptive effects (time course) produced by morphine and metamizol on nociception level I are shown either alone or in combination. The antinociception produced by the combination represents potentiation of antinociceptive effects; likewise, both the time course and AUC obtained with this combination were higher

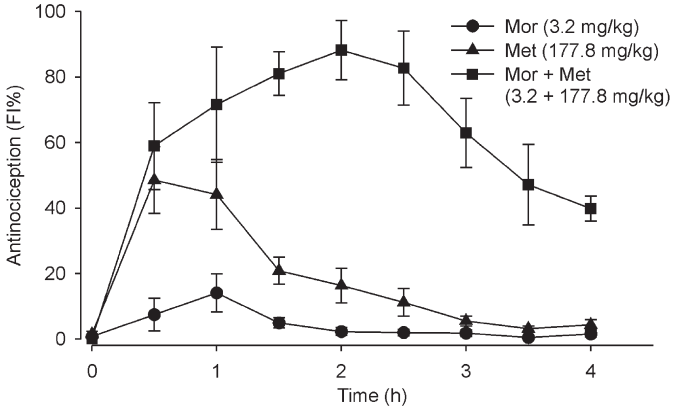

Fig. 3. Antinociceptive effects (time course), expressed as the recovery of the functionality index (FI%) produced by either morphine (Mor), metamizol (Met), or the combination of morphine and metamizol (Mor+Met) at nociception level I. The *y*-axis depicts the antinociceptive effect in FI%. The *x*-axis shows the time in hours. Data are expressed as mean±SEM of six determinations. The combination produced a clear potentiation of the antinociceptive effects ($P<0.001$). Metamizol showed an AUC of 76.3±9.9 au; morphine showed an AUC of 17.0±3.5 au. In contrast, the combination presented an AUC of 263.7±28.6 au during all observation periods (4 h).

($P<0.01$) than the AUC obtained by the sum of AUC individuals. It is noteworthy that the antinociceptive effect (39.8±3.9%) was presented with the combination at the end of the experiment (4 h); in contrast, either morphine or metamizol alone did not produce significant antinociceptive effects at that time. The combination showed better antinociceptive coverage through time than did any individual analgesic drugs or the expected theoretical sum.

The AUC reflects the global antinociceptive effect within the time of evaluation (4 h). As seen in Fig. 4, there were no significant differences between the antinociceptive effects of the single dose of morphine and metamizol when studied at different levels of nociception. However, the morphine and metamizol combination produced AUCs that were significantly different from those produced for either individual drugs or the sum of the individual effects. The combination of morphine with metamizol produced a potentiated antinociceptive effect at the different levels of nociception evaluated using the PIFIR model. It was equally effective on levels I (256.2±30.9 au) and II (248.6±14.3 au) and less effective on level III (165.9±24.2 au). At nociceptive level I, the combination of morphine and metamizol yielded a significantly greater (174.6%) antinociceptive effect than expected from the sum of the individual effects. It is, therefore, possible to establish that the combination (morphine and metamizol) produced potentiation of antinociceptive effects at nociception level I. The antinociceptive effect of the combination of morphine and metamizol observed at nociceptive level II was increased by 361.2% ($P<0.01$) compared to the theoretical sum of the individual effects. It represents a potentiation of the antinociceptive activity of morphine and metamizol. Finally, in the intense nociception experimental condition (level III), the antinociceptive effect observed using the combination was significantly greater ($P<0.05$) than that expected on the basis of the addition of the individual effects. The antinociceptive effect was 108.2% greater than the theoretical sum, which gives evidence of potentiation of the antinociceptive effects at nociception level III. The combination was less effective at nociception level III, but the antinociceptive effect produced is very useful when compared with the antinociceptive effects produced by the analgesic drugs administered alone. Morphine produced 40.7±9.5 au, whereas metamizol produced 39.1±14.2 au.

Fig. 5 shows in bars the maximum effect (Emax) determined in the time course of the antinociceptive effects of morphine, metamizol, and the combination at nociception levels I, II, and III. Also, the maximum effect corresponding to the theoretical sum of the individual effects of morphine and metamizol is included. At the three different levels of nociception, the Emax from the time courses for morphine, metamizol, or combinations are not significantly different. The antinociceptive effects, expressed as the Emax of the morphine and metamizol combination, were significantly greater than those antinociceptive effects produced by either morphine alone at nociception levels I and II or metamizol alone at nociception levels II and III. The antinociceptive effects observed using the combination were not significantly different than those expected on the basis of the addition of the individual effects. When the Emax values were analyzed, the combinations showed additive effects at nociception levels I, II, and III. The combination produced additive antinociceptive effects (Emax) at the different levels of nociception evaluated using the PIFIR model, and it was equally effective at levels I (88.2±9.1%), II (73.2±10.1%), and III (75.1±8.9%).

Fig. 6 shows the antinociceptive effect determined in each time course 2 h after administration of the analgesic drugs alone or in

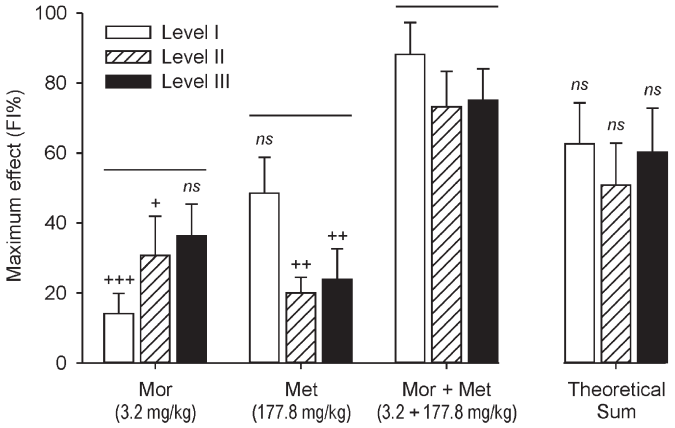

Fig. 5. Antinociceptive effects of morphine, metamizol, and the combination of morphine and metamizol (expressed as maximum effect of the corresponding time course) as well as the theoretical sum of the individual effects determined at the three levels of nociception. Bars represent the mean±SEM of six determinations. Horizontal lines over bars indicate no significant (ns) difference between groups. +, ++, and +++ indicate $P<0.05$, 0.01, and 0.001, respectively, versus response with the combination from a Tukey test. ns:$P>0.05$ comparing combination data versus theoretical sum with a Student's *t*-test with Bonferroni correction. The type of observed antinociceptive interaction is addition.

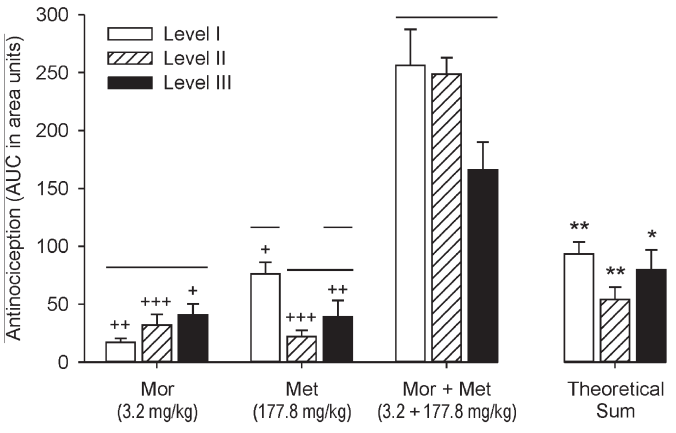

Fig. 4. Antinociceptive effects of morphine, metamizol, and the combination of morphine and metamizol as well as the theoretical sum of the individual effects (expressed as the area under the curve within the time of evaluation (4 h)) determined at the three levels of nociception. Bars represent the mean±SEM of six determinations. Horizontal lines over bars indicate no significant (*ns*) difference between groups. +, ++, and +++ indicate $P<0.05$, 0.01, and 0.001, respectively, versus response with the combination from a Dunnett T3 test. * and ** indicate $P<0.05$ and $P<0.01$, respectively, for comparing combination data versus the theoretical sum using a Student's *t*-test with Bonferroni correction. The type of observed antinociceptive interaction is potentiation.

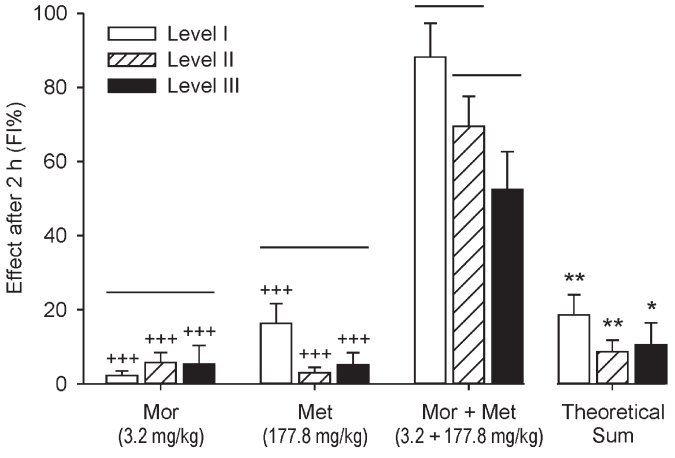

Fig. 6. Antinociceptive effects of morphine, metamizol, and the combination of morphine + metamizol (antinociceptive effects produced 2 h after administration) as well as the theoretical sum of the individual effects determined at the three levels of nociception. Bars represent the mean±SEM of six determinations. Horizontal lines over bars indicate no significant (ns) difference between groups. +++ indicates $P<0.001$ versus response with the combination from a Tukey test. * and ** indicate $P<0.05$ and $P<0.01$, respectively, for comparison of combination data and theoretical sum by a Student's *t*-test with Bonferroni correction. The type of observed antinociceptive interaction is potentiation.

combination. The effect after 2 h corresponding to the "Theoretical Sum" of the individual effects of morphine and metamizol was also included. At the three different levels of nociception, the effects 2 h after administration (determined from the time course) of morphine or metamizol do not differ significantly. This figure also shows a significantly greater effect of the combinations than the individual compounds. The antinociceptive effects observed using the combinations were significantly greater than those expected on the basis of the addition of the individual effects. The antinociceptive effects were 374.2%, 698.8%, and 399.0% greater than the "Theoretical Sums" at nociception levels I, II, and III, respectively. The combination produced potentiation of the antinociceptive effects at each different level of nociception. The combination produced potentiating antinociceptive effects at the different levels of nociception evaluated when the effects 2 h after drug administration were considered. The combinations showed the following antinociceptive effects: 88.2±9.1% at nociception level I, 69.5±8.1% at level II, and 52.4±10.2% (less effective) at level III. The combination was less effective at level III, but the antinociceptive effects produced are useful in comparison to those produced by the analgesic drugs administered alone. Morphine produced an effect of 5.4±5.0%, whereas metamizol produced an effect of 5.2±3.2%.

4. Discussion

The purpose of this work was to analyze a combination (morphine 3.2 mg/kg and metamizol 177.8 mg/kg) previously described as optimal for producing maximal antinociceptive effects (potentiation) in the PIFIR model (López-Muñoz, 1994) at three different levels of nociception now. The administration of different levels of uric acid showed different changes in the FI%, and there was a spontaneous recovery of FI% in rats receiving 30% uric acid 7 h after its administration. There was spontaneous recovery of the FI% in rats receiving 50% uric acid six days after its administration, and there was spontaneous recovery in rats receiving 50%×2 uric acid after 14 days. These data show that increasing the concentration of the uric acid in the suspension administered reduced the time for which functional loss was recorded. Spontaneous recovery was delayed animals treated with higher concentrations of uric acid, and the dysfunction lasted longer without analgesic treatment (i.e., when nociception was increased).

Acetylsalicylic acid was presented because it is a prototype analgesic agent and a good pharmacological example of an analgesic drug at the three levels of nociception. The doses used for obtaining the DRCs of acetylsalicylic acid were selected due to their lack of adverse effects when administered alone. They were calculated on an increasing 0.25 logarithmic unit basis. Acetylsalicylic acid showed a significant modification in its antinociceptive effect dependent of the level of nociception. The same dose of acetylsalicylic acid (562.3 mg/kg) produced different antinociceptive effects at diverse levels of nociception (or pain). When the different experimental conditions of uric acid administration are used in the PIFIR model, diverse dysfunction, spontaneous recovery, and antinociceptive efficacy for the same analgesic dose are observed (López-Muñoz, 1986). This provides the basis of the three different levels of nociception in the PIFIR model.

Preclinical studies had shown that metamizol increases morphine-induced antinociception when these drugs are co-administered. The observed effects are synergistic in pain models such as the PIFIR model (López-Muñoz, 1994; Domínguez et al., 2000), writhing test (Taylor et al., 1998), and tail flick (Hernández-Delgadillo et al., 2003). It has also been shown that the administration of metamizol before abdominal surgery diminishes morphine consumption in humans (Tempel et al., 1996). Our study showed that the co-administration morphine (3.2 mg/kg s.c.) with metamizol (177.8 mg/kg s.c.) had important antinociceptive effects at the three levels of nociception used in the PIFIR model. In the three cases, it produced a synergistic interaction and potentiation of the individual effects. Although the combination was useful at the three levels of nociception, it was not equally effective in all cases. These results are in agreement with those presented by López-Muñoz (1986). This work demonstrated that no significant differences between the DRC of metamizol exist at nociception levels I and II, but it suggested significant differences at level III of nociception. Using the same experimental conditions, paracetamol, indomethacin, and acetylsalicylic acid showed differences in their respective DRCs (Fig. 2). The results of this study are, therefore, important because they show that the combination is useful at the three conditions of nociception even though the individual analgesic drugs exhibited the same therapeutic utility. All of this evidence shows that antinociceptive potentiation between morphine and metamizol occurs for a wide variety of noxious stimuli and different intensities of nociception.

One explanation for these results obviously depends of the intensity of nociception, but the different mechanisms of action involved for each analgesic drug are another important factor. Morphine and other opioid medications interact with the opioid receptors and produce analgesia by the same mechanism of action as the encephalins. Additionally, morphine can interact with local opioid receptors in supraspinal structures to activate the supraspinal system (Martin, 1984; Lipp, 1991). Some evidence supports a role for the activation of the serotonergic and noradrenergic inhibitory routes at the bulbospinal level (Kawamata et al., 1993). Additionally, metamizol has a direct effect on hyperalgesic inflammatory states (Lorenzetti and Ferreira, 1985). It is known that metamizol and other NSAID analgesics inhibit the synthesis of prostaglandins at the central as well as peripheral levels (Campos et al., 1999). Another mechanism of action involved in the antinociceptive effect of metamizol is the activation of the NO-cGMP peripherally (Duarte et al., 1992). It has also been suggested that glutamate may interfere with metamizol at the central level of nociceptive activity (Beirith et al., 1998).

Christie et al. (2000) tried to provide a mechanism explaining the interaction between opioids and NSAIDs at nerve terminals in the central nervous system. Opioids acting on μ-opioid receptors inhibit neurotransmitter release by stimulating PLA_2 via Gi-proteins. This leads to the formation of the 12-lipoxygenase metabolites of arachidonic acid, which enhance the activity of voltage-dependent K^+ channels that inhibit neurotransmitter release. Cyclo-oxygenase inhibitors block alternative pathways of arachidonic acid metabolism, and metamizol preferentially inhibits COX-2 (Campos et al., 1999). This shunts the enhanced formation of 12-lipoxygenase, which enhances the efficacy of opioids to inhibit the probability of release from GABAergic nerve terminals; therefore, this process disinhibits the descending antinociceptive pathways. This mechanism can account for opioid and NSAID synergism as well as the naloxone-sensitive analgesic actions of NSAIDs in the CNS (Christie et al., 2000). Although the peripheral effects of NSAIDs are well known, metamizol produces antinociception by acting upon central nervous system structures (Tortorici and Vanegas, 2000). These central effects can be reduced by naloxone, suggesting that the endogenous opioid system is involved (Tortorici et al., 1996). There are experimental arguments supporting the idea that endogenous opioids are released as a result of using the combination of morphine with metamizol. For example, a dose of naloxone that is completely effective for blocking morphine does not block the effects of the combination, while a higher naloxone dose does. This suggests that there are more opioids to antagonize in the latter than in the former situation (Hernández-Delgadillo and Cruz, 2006). Naloxone can cross blood brain barrier, so it is possible that this selective antagonist blocked the antinociceptive effects of the combination at peripheral and central levels. Other possible mechanisms to explain the potentiation of morphine antinociception by metamizol involve the participation of the L-arginine-NO-cyclic GMP pathway. Local pretreatment with N^G-L-nitro-arginine methyl ester (L-NAME, an inhibitor of nitric oxide (NO) synthesis) but not D-NAME (inactive isomer of L-NAME) provided dose-dependent blockade of the antinociception produced by the local administration of the morphine and metamizol combination. This

suggests that the potentiation of the morphine effect by metamizol is due, at least in part, to a local release of NO. This also suggests that the activation of the peripheral NO-cyclic GMP pathway plays an important role in the antinociception produced by the combination (Duarte et al., 1992).

A limiting factor in the clinical use of opioid analgesic drugs is the development of analgesic tolerance after repeated administration. The combination morphine with metamizol has been demonstrated to be an effective therapeutic strategy in the management of pain even when tolerance to morphine exists. In previous studies, it was observed that metamizol (via repeated administration of the morphine + metamizol combination) potentiates morphine antinociception after chronic treatment in both rats injected with uric acid into the right knee joint as well as rats evaluated in the tail flick test (Domínguez et al., 2000; Hernández-Delgadillo et al., 2003). In both cases, the analgesic drugs were administered systemically. Also, metamizol potentiates morphine-induced antinociception in metamizol-treated as well as morphine-tolerant rats (Hernández-Delgadillo et al., 2003). These results contrast with those reported by Tortorici and Vanegas (2000), however, who suggest that morphine lacked an effect when given to rats rendered tolerant to metamizol by repeated injections into the periaqueductal grey. Another adverse effect that opioids present is the inhibition of gastrointestinal transit. Experimental analysis of these adverse effects obtained in our laboratory has shown that the combination (morphine and metamizol) inhibits gastrointestinal transit at a level similar that of morphine alone, suggesting that metamizol does not potentiate the constipation induced by morphine (Hernández-Delgadillo et al., 2002).

In summary, the combination of morphine (3.2 mg/kg) with metamizol (177.8 mg/kg) produced potentiating antinociceptive effects at different levels of nociception or pain using the PIFIR model. The combination was equally effective at levels I and II and less effective at level III. These results have potential therapeutic usefulness in the treatment of pain.

Acknowledgements

We wish to thank M. De la O-Arciniega, A. Huerta, L. Oliva, and F. Sánchez for technical assistance. B Godínez-Chaparro and JC Huerta-Cruz are National Council for Sciences and Technology (CONACYT) fellows, México.

References

Beirith A, Santos ARS, Rodrigues ALS, Creczynski-Pasa TB, Calixto JB. Spinal and supraspinal antinociceptive action of dipyrone in formalin, capsaicin and glutamate tests. Study of the mechanism of action. Eur J Pharmacol 1998;345:233–45.

Campos C, de Gregorio R, García-Nieto R, Gago F, Ortiz P, Alemany S. Regulation of cyclooxygenase activity by metamizol. Eur J Pharmacol 1999;378:339–47.

Chandrasekharan NV, Dai H, Roos KL, Evanson NK, Tomsik J, Elton TS, et al. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proc Natl Acad Sci USA 2002;99:13926–31.

Christie MJ, Connor M, Vaughan CW, Ingram SL, Bagley EE. Cellular actions of opioids and other analgesics: implications for synergism in pain relief. Clin Exp Pharmacol Physiol 2000;27:520–3.

Covino BG, Dubner R, Gybels J, Kosterlitz HW, Liebeskind JC, Sternbach RA, et al. Ethical standards for investigation of experimental pain in animals. Pain 1980;9:141–3.

Domínguez RAM, Hernández DGP, Ventura MR, Díaz RMI, López-Muñoz FJ. Analgesic efficacy of the combination metamizol + morphine after subchronic treatment in rat. Drug Dev Res 2000;51:260–7.

Duarte ID, dos Santos IR, Lorenzetti BB, Ferreira SH. Analgesia by direct antagonism of nociceptor sensitization involves the arginine–nitric oxide–cGMP pathway. Eur J Pharmacol 1992;217:225–7.

García-Alonso F, González MJ, López-Alvarez M, Paliop R. Comparative study of the efficacy of dipyrone, diclofenac sodium and pethidine in acute renal colic. Eur J Clin Pharmacol 1991;40:543–6.

García-Hernández L, Déciga-Campos M, Guevara-López U, López-Muñoz FJ. Co-administration of rofecoxib and tramadol results in additive or sub-additive interaction during arthritic nociception in rat. Pharmacol Biochem Behav 2007;87:331–40.

Gutstein HB, Akil H. Opioid analgesics. In: Brunton LL, Lazo JS, Parker KL, editors. Goodman & Gilman's the pharmacological basis of therapeutics. eleventh ed. USA: McGraw-Hill; 2006. p. 547–90.

Hernández-Delgadillo GP, Cruz SL. Endogenous opioids are envolved in morphine and dipyrone analgesic potentiation in the tail flick test in rats. Eur J Pharmacol 2006;546:54–9.

Hernández-Delgadillo GP, López-Muñoz FJ, Salazar LA, Cruz SL. Morphine and dipyrone co-administration delays tolerance development and potentiates antinociception. Eur J Pharmacol 2003;469:71–9.

Hernández-Delgadillo GP, Ventura MR, Díaz RMI, Domínguez RAM, López-Muñoz FJ. Metamizol potentiates morphine antinociception but not constipation after chronic treatment. Eur J Pharmacol 2002;441:177–83.

Hertle L, Nawrath H. Effect of baralgin on isolated preparations of the upper urinary tract in man. Urol Int 1984;39:84–90.

Kawamata M, Omote K, Namiki A, Ishitani K. Contribution of descending inhibitory and spinal cholinergic systems to visceral antinociception of morphine. Anesthesiology 1993;79:A701.

Lipp J. Possible mechanism of morphine analgesia. Clin Neuropharmacol 1991;14:131–47.

López-Muñoz, F.J. Análisis de algunos factores que determinan la farmacodinamia de agentes analgésicos. Thesis for obtainning Master in Science degree, Department of Pharmacology and Toxicology, Cinvestav. México D.F. México;1986.

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

López-Muñoz FJ, Salazar LA, Castañeda-Hernández G, Villarreal JE. A new model to assess analgesic activity: Pain-induced functional impairment in the rat (PIFIR). Drug Dev Res 1993;28:169–75.

López-Muñoz FJ, Villalón CM, Terrón JA, Salazar LA. Analgesic interactions produced by dipyrone and either morphine or D-propoxyphene in the pain-induced functional impairment model in rat. Drug Dev Res 1994;32:50–7.

López-Muñoz FJ, Díaz-Reval MI, Terrón JA, Déciga M. Analysis of the analgesic interactions between ketorolac and tramadol during arthritic nociception in rat. Eur J Pharmacol 2004;484:157–65.

Lorenzetti BB, Ferreira SH. Mode of analgesic action of dipyrone: direct antagonism of inflammatory hyperalgesia. Eur J Pharmacol 1985;114:375–81.

MacPherson RD. New directions in pain management. Drugs Today (Barc) 2002;38:135–45.

Martin WR. Pharmacology of opioids. Pharmacol Rev 1984;35:283–323.

Martin TJ, Eisenach JC. Pharmacology of opioid and nonopioid analgesics in chronic pain states. J Pharmacol Exp Ther 2001;299:811–7.

Pompeia C, Boaventura MF, Curi R. Antiapoptotic effect of dipyrone on HL-60, Jurkat and Raji cell lines submitted to UV irradiation, arachidonic acid and cycloheximide treatments. Int Immunopharmacol 2001;1:2173–82.

Raffa RB. Pharmacology of oral combination analgesics: rational therapy for pain. J Clin Pharm Ther 2001;26:257–64.

Reis GML, Doretto MC, Duarte IDG, Tatsuo MAKF. Do endogenous opioids and nitric oxide participate in the anticonvulsant action of dipyrone? Braz J Med Biol Res 2003;36:1263–8.

Ripamonti C, Dickenson ED. Strategies for the treatment of cancer pain in the new millennium. Drugs 2001;61:955–77.

Rowland, M., Tozer, T.N. Clinical Pharmacokinetics: concepts and applications. Second ed. Philadelphia, London, Lea & Febiger; 1989. p. 255–257, p. 459–63.

Sanchez S, Alarcon de la Lastra C, Ortíz P, Motilva V, Martin MJ. Gastrointestinal tolerability of metamizol, acetaminophen, and diclofenac in subchronic treatment in rats. Dig Dis Sci 2002;47:2791–8.

Seegers AJM, Jager LP, Zandberg P, van Noordwijk J. The anti-inflammatory, analgesic and antipyretic activities of non-narcotic analgesic drug mixtures in rats. Arch Int Pharmacodyn 1981;251:237–54.

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

Tempel G, von Hundelshausen B, Reeker W. The opiate-sparing effect of dipyrone in post-operative pain therapy with morphine using a patient-controlled analgesic system. Intensive Care Med 1996;22:1043–7.

Tortorici V, Vanegas H. Opioid tolerance induced by metamizol (dipyrone) microinjections into the periaqueductal gray of rats. Eur J Neurosci 2000;12:4074–80.

Tortorici V, Vasquez E, Vanegas H. Naloxone partial reversal of the antinociception produced by dipyrone microinjected into the periaqueductal gray of rats. Possible involvement of medullary off- and on-cells. Brain Res 1996;725:106–10.

Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–10.