Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 469 (2003) 71–79
www.elsevier.com/locate/ejphar

Morphine and dipyrone co-administration delays tolerance development and potentiates antinociception

Gloria P. Hernández-Delgadillo, Francisco J. López-Muñoz, Luis A. Salazar, Silvia L. Cruz*

Departamento de Farmacobiología, Cinvestav, IPN, Calzada de los Tenorios No. 235, Col. Granjas Coapa, 14330, México D.F., Mexico

Received 27 January 2003; received in revised form 10 April 2003; accepted 15 April 2003

Abstract

This work analyses the time course of tolerance development and antinociceptive potentiation throughout repeated co-administration of morphine (an opioid receptor agonist) plus dipyrone (a non-steroidal anti-inflammatory drug) in the tail-flick test. Male Wistar rats were i.v. injected with morphine (3.1 mg/kg), dipyrone (600 mg/kg) or the combination morphine/dipyrone twice a day for 5 days. Dipyrone produced antinociceptive effects with a trend towards tolerance development at the end of the treatment. Morphine was initially effective, but complete tolerance developed after its fifth administration. The combination of morphine plus dipyrone produced a significant potentiation and longer duration of antinociceptive effects. The antinociceptive efficacy of morphine and dipyrone co-administration gradually decreased after the sixth injection. An additional group of rats treated with dipyrone for 11 days developed complete tolerance after the 19th administration. These data suggest that repeated co-administration of morphine plus dipyrone results in a delay of tolerance development and in a potentiation of their individual antinociceptive effects.

Keywords: Opioid; Metamizol; Antinociception; Potentiation; Tolerance

1. Introduction

Morphine is still the drug of choice for the treatment of moderate to severe pain (Martin and Eisenach, 2001). In spite of this, the usefulness of opioids in chronic pain management is limited because tolerance to their analgesic effects develops after repeated administration (Bhargava, 1994; Gutstein and Akil, 2001) which leads to a gradual drug dose escalation and the increase of undesirable effects associated with it (Mercadante, 1999).

A strategy to reduce the unwanted side effects of high doses of analgesics is to combine low doses of opioids and non-steroidal anti-inflammatory drugs (Lasagna, 1965; Picard et al., 1997). This approach not only reduces the risks associated with the use of high doses of individual drugs, but sometimes results in improved analgesic treatments (Grotto et al., 1965; Maves et al., 1994; Christie et al., 1999). In this regard, extensive pre-clinical and clinical research in the field of pain relief has shown that the acute combination of opioids with non-steroidal anti-inflammatory drugs produces analgesic potentiation (Calimlim et al., 1976; Bentley and Head, 1987; Malmberg and Yaksh, 1993; World Health Organization, 1986; Sandrini et al., 1998, 1999; Lashbrook et al., 1999). In contrast, little is known about the antinociceptive effects of repeated administration of combined analgesic treatments.

Among non-steroidal anti-inflammatory drugs, dipyrone (also known as metamizol) is widely used in Latin America, Germany and other European countries (Miralles et al., 1987; Garcia-Alonso et al., 1991) due to its high analgesic efficacy and good gastric tolerability (Patel et al., 1980; Rodriguez et al., 1994; Planas et al., 1998). Some pre-clinical reports have shown that, acutely, dipyrone enhances morphine-induced antinociception (Carlsson and Jurna, 1987; Lopez-Muñoz, 1994; Taylor et al., 1998; Aguirre-Bañuelos and Granados-Soto, 1999). In a recent work using an inflammatory nociception test, we found that the combination of morphine plus dipyrone produced analgesic potentiation not only in acutely treated rats, but also in animals that had been treated once a day for 12 days with this combination (Hernandez-Delgadillo et al., 2002) without producing an increase in constipation effects. Due to limitations associated with the model used in

* Corresponding author. Tel.: +52-55-5483-2853; fax: +52-55-5483-2863.
E-mail address: cruz_farma@yahoo.com (S.L. Cruz).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01727-8

that work, we only evaluated two time points, at the beginning and at the end of the experimental protocol, using independent groups of animals (i.e., one group received the combination of morphine plus dipyrone once while the other received the same combination for 12 times and was evaluated at the end of the treatment).

The purpose of the present work was to study the time course of tolerance development and antinociceptive potentiation throughout repeated administration of a combined treatment of morphine plus dipyrone in the tail-flick test. This model was selected because it allows repeated antinociceptive evaluation in the same group of animals for a relatively prolonged period of time (Nance and Sawynok, 1987).

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 180 to 220 g, were used. Animals were housed in an animal room with controlled temperature (22 ± 2 °C) under a 12:12-h light–dark cycle (lights on at 7:00 h), with free access to drinking water and commercial food. In order to reduce stress, all rats were handled twice a day for 2 days before testing the drugs. The local Committee on Ethics on Animal Experimentation approved all experimental procedures, which followed the regulations established in the Mexican official norm for the use and care of laboratory animals ''NOM-062-ZOO-1999''. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983).

2.2. Drugs

Morphine sulphate was obtained from Laboratorios Pisa (Mexico City, Mexico). Dipyrone sodium was purchased from Hoechst (Mexico City, Mexico). Heparine sodium was bought from Sigma (St. Louis, MO, USA). All drugs were dissolved in sterile saline solution and administered intravenously.

2.3. Surgical procedure and drug administration

Drugs were administered through a permanent cannula placed into the right jugular vein. In order to place the cannula, rats were briefly anaesthetised with ether and, afterwards, a polyethylene catheter (PE50), flushed with heparine solution (500 units/ml), was inserted and fixed into the right jugular vein. The distal end of the catheter was guided subcutaneously to the top of the neck, where it was exteriorised and sealed with a metal plug. After surgery, rats were individually housed and allowed for a 24-h recovery period. For drug administration, a 24-gauge stainless steel needle attached to a 5-ml Becton Dickinson syringe was inserted into the outer tip of the jugular cannula. Drugs were injected in a volume of 1 ml during 2 min, using an infusion pump (KD Scientific, USA). After each drug injection, the catheter was flushed with heparine solution in a volume that exceeded the estimated catheter dead space.

2.4. Evaluation of antinociceptive activity

A standardised tail-flick apparatus (UGO BASILE, Italy), with a radiant heat source connected to an automatic timer was used to assess the antinociceptive response. In this model, antinociception is seen as an increase in the latency to tail withdrawal (D'Amour and Smith, 1941; modified by Nance and Sawynok, 1987). At the beginning of the study, the stimulus intensity was adjusted so that the baseline tail-flick latency was 6.0 ± 0.5 s. Before the first drug injection, animals were screened for thermal nociception. Rats showing no flicking within 5.5 to 6.5 s (approximately 10–15% of the total) were excluded. The cut-off time in the absence of response was set at 15 s to avoid tail skin tissue damage. For each rat, the mean baseline latency derived from two tests was obtained before each drug injection. After drug administration, tail withdrawal latency was determined every 15 min for the first hour and every 30 min for the subsequent 2 h. Rats were euthanised at the end of the experiments with carbon dioxide.

2.5. Study design

2.5.1. Protocol 1: dose–response curves

Eight independent groups of rats ($n = 8$, each) were used to complete dose–response curves for morphine (1, 1.8, 3.1, 5.6 and 10 mg/kg) and dipyrone (400, 600 and 1000 mg/kg) in order to select the doses of individual antinociceptive drugs to be used in chronic combination studies.

2.5.2. Protocol 2: repeated administration of antinociceptive drugs

Four additional independent groups of rats ($n = 8$, each) were administered with 3.1 mg/kg morphine, 600 mg/kg dipyrone, the combination of the same doses of morphine plus dipyrone, or saline solution twice a day (at 9:00 and 17:00 h) for 5 days. Animals were tested for antinociceptive effects after each administration as previously described. The combination morphine/dipyrone was given in a single injection in a total volume of 1 ml.

An additional group of rats ($n = 8$) was administered with 600 mg/kg dipyrone twice a day for 11 days (see below). To avoid tissue damage due to an excessive exposure to the thermal stimulus in this group, the latency to tail withdrawal was determined only after morning injections.

2.6. Data and statistical analysis

All results are presented as the mean ± S.E.M. of eight determinations. Antinociception was evaluated by three

different parameters: (a) an increase in the latency to tail withdrawal; (b) the percentage of maximum possible effect; and (c) the area under the curve for each time course. The percentage of maximum possible effect (%MPE) was calculated at the peak effect, using the formula $\%MPE=[(A-B)/(15-B)]\times 100$, where B and A were the tail-flick latencies before and after drug administration, and 15 was the cut-off time value. The area under the curve was calculated by the trapezoidal rule (Gibaldi, 1991). To determine the half-life of the decay of antinociceptive effects in each time course, the experimental points were adjusted by an exponential equation. A linear regression was used to evaluate trends in the development of tolerance. A one-way analysis of variance (ANOVA) for repeated measures, followed by Dunnett's test, was used to compare the drug effects after repeated administrations with respect to the effects observed with the first injection. A two-way ANOVA (administration, treatment, and interaction) followed by a Tukey test was applied for comparisons between the observed effects of the combination of morphine plus dipyrone and the expected sum of individual effects at each administration. The expected values were calculated on the basis of addition of the effects of the individual component drugs (Seegers et al., 1981; Hernandez-Delgadillo et al., 2002). The mean responses of two independent experimental groups were compared using a Mann–Whitney test. The program used to perform statistical procedures was SigmaStat (version 2.03, Jandel Scientific). The dose–response curve adjustment was done with the Win-nonlin program (Pharsight, version 2.1).

3. Results

3.1. Selection of doses for combination studies

Fig. 1 shows the dose–response curves of the antinociceptive effects of morphine and dipyrone, expressed as the percentage of maximum possible effect. Both morphine and dipyrone produced dose-dependent antinociceptive effects, being morphine at least 250 times more potent than dipyrone. The calculated ED_{50} value for morphine after sigmoideal curve fitting was 2.54 ± 0.04 mg/kg. The graphically estimated ED_{50} of dipyrone was approximately 630 mg/kg. This curve could not be completed and adjusted because doses higher than 1000 mg/kg produced muscle rigidity, a toxic effect of dipyrone. Based on these data, we selected a dose of each drug that was close to its ED_{50} value in order to observe synergistic effects when combined. The dose of morphine was chosen so that it would be high enough to induce a clear development of tolerance after repeated administration (Cox and Tiffany, 1997). Thus, the selected doses of morphine and dipyrone were 3.1 and 600 mg/kg, respectively.

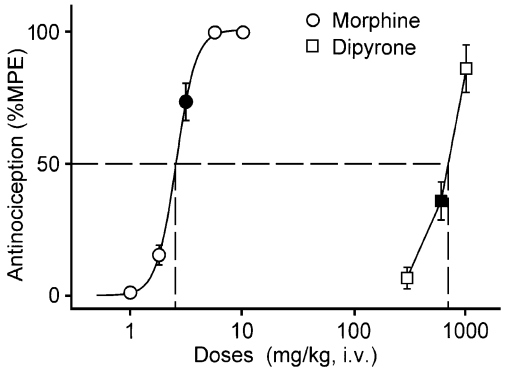

Fig. 1. Dose–response curves for morphine and dipyrone in the tail-flick test in rats. The parameter evaluated was the latency to tail-flick withdrawal as a function of dose. Antinociceptive effects are expressed as the percent of maximum possible effect (%MPE). Dashed lines represent the approximated points of their respective ED_{50} values. Black symbols show the doses selected for repeated administration studies. Each point represents the mean ± S.E.M. of eight determinations.

3.2. Time course of antinociceptive effects

Fig. 2 shows the time course of the antinociceptive effects of 10 administrations (twice a day for 5 days) of dipyrone, morphine, and the combination of morphine plus dipyrone. Data corresponding to saline-treated rats are also shown. Repeated administration of saline solution did not modify the latency to tail withdrawal along the 3 h of experimental evaluation.

As seen in all panels, the basal latency value before the first administration of any treatment was close to 6 s. Dipyrone produced a slight increase in the tail-flick latency that was maximal at 30 min after injection and returned to basal values after approximately 1.5 h. The same pattern was seen after each dipyrone administration. The first dose of morphine was more effective than any dose of dipyrone, but subsequent administrations of this opioid receptor agonist resulted in a gradual decrease of the maximal peak effect with complete tolerance occurring from the sixth administration onwards. Whenever effective, morphine reached its peak effect within the first 15-min post-injection. The first five administrations of morphine plus dipyrone induced an increase in the tail-flick latency that achieved the cut-off time (15 s) within 15 min, remained stable for the next 45 min and then gradually decreased towards baseline values. The maximal effect decreased with subsequent administrations, indicating that tolerance developed. In general, the antinociceptive effects of the combination of morphine plus dipyrone lasted longer than those effects of individual drugs. Finally, it is important to mention that basal latency in animals treated chronically with drugs, measured immediately before each drug administration, was stable throughout the study indicating that neither tissue damage nor hyperalgesia occurred.

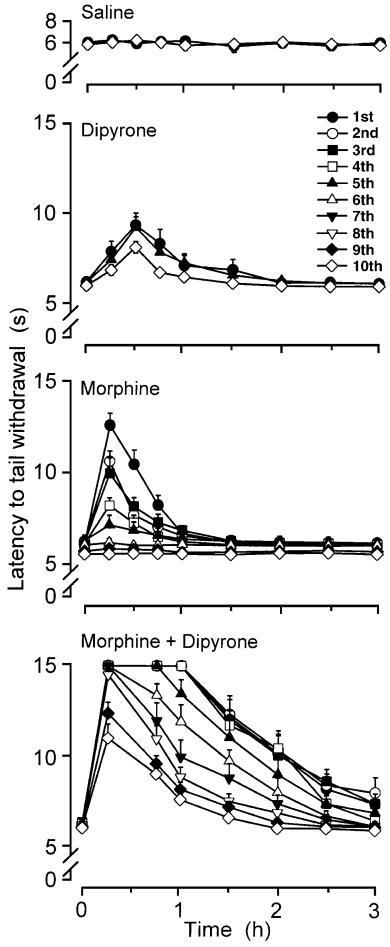

Fig. 2. Time courses of the antinociceptive effects of repeated administration of saline solution, 600 mg/kg dipyrone, 3.1 mg/kg morphine, or the combination of the same doses of morphine plus dipyrone measured as an increase in the tail-flick latency. For reasons of clarity, only the curves corresponding to representative administrations are shown in saline- and dipyrone-treated groups. Each point represents the mean ± S.E.M. of eight rats.

The shape of the time course curves corresponding to the effects of morphine plus dipyrone strongly suggests that limitations given by the cut-off value resulted in an artificial plateau response for the first five administrations. As an approach to overcome this limitation, an exponential curve was adjusted to the decay phase of the experimental curves using the points obtained after the plateau phase (i.e., the points corresponding to the effects observed at 1 h and later). This analysis was also conducted for the time courses corresponding to the effects of the individual drugs in order to obtain an estimated theoretical maximal effect and the half-life of the decay of antinociceptive responses. For the curves corresponding to morphine and dipyrone alone, we used all the experimental points from the maximal effect to the end of the observation time. The results are summarised in Table 1. As shown, the time to peak was shorter for morphine and the combination of morphine plus dipyrone than for dipyrone. The experimental maximal effects observed at the first and last effective administrations of dipyrone and morphine alone were very similar to those calculated after exponential adjustment. In contrast, the calculated maximal effect of the combination morphine/dipyrone was significantly higher than the experimental value obtained for the first drug administration (33.6 ± 5.1 vs. 15 s; $P < 0.001$; Mann–Whitney test), but not for the last administration (12.6 ± 1 vs. 11 ± 0.8, n.s.). As to the duration of the effects, the antinociception produced by dipyrone lasted longer than that produced by morphine. The estimated half-life of the antinociceptive effects of both drugs was relatively constant throughout repeated administrations, i.e., it did not change from the first to the last administration. The half-life of the effect of the combination of morphine plus dipyrone based on the estimated maximal effect at the first administration was approximately 1 h.

3.3. Tolerance development

For the study of the antinociceptive effects of dipyrone, morphine, and the combination of morphine plus dipyrone, we also compared the area under the curves obtained from the time courses presented in Fig. 2. The advantage of using this parameter is that the results obtained during the 3 h of experimental evaluation can be taken into account. The results are shown in Fig. 3. Linear regressions (continuous lines) were used to determine trends in the development of tolerance. As shown, the effects of dipyrone were relatively constant throughout the 10-administration treatment, since a one-way ANOVA for repeated measures did not reveal statistically significant differences for the antinociception seen after each administration ($F(9,63) = 1.1$,

Table 1
Parameters of the time course curves of the antinociceptive effects of 600 mg/kg dipyrone, 3.1 mg/kg morphine, and the combination of the same doses of morphine and dipyrone (M + D)

Drugs	t_{Emax} (h)[a]	Maximal effect (Latency, s)					
		Experimental		Calculated		Half-life (h)	
		First ad.	Last ad.[b]	First ad.	Last ad.	First ad.	Last ad.
Dipyrone	0.50	9.36 ± 0.66	8.13 ± 0.32	9.65 ± 0.85	7.84 ± 0.23	0.81 ± 0.11	0.71 ± 0.06
Morphine	0.25	12.60 ± 0.65	7.14 ± 0.51	13.95 ± 1.00	7.39 ± 0.32	0.53 ± 0.04	0.60 ± 0.07
M + D	0.25	15.00 ± 0.00	11.03 ± 0.78	33.55 ± 5.14	12.58 ± 1.04	1.00 ± 0.22	0.75 ± 0.07

[a] Time to maximal effect.
[b] For dipyrone and the combination of M + D, the last effective administration (last ad.) was the 10th, for morphine, it was the 5th.

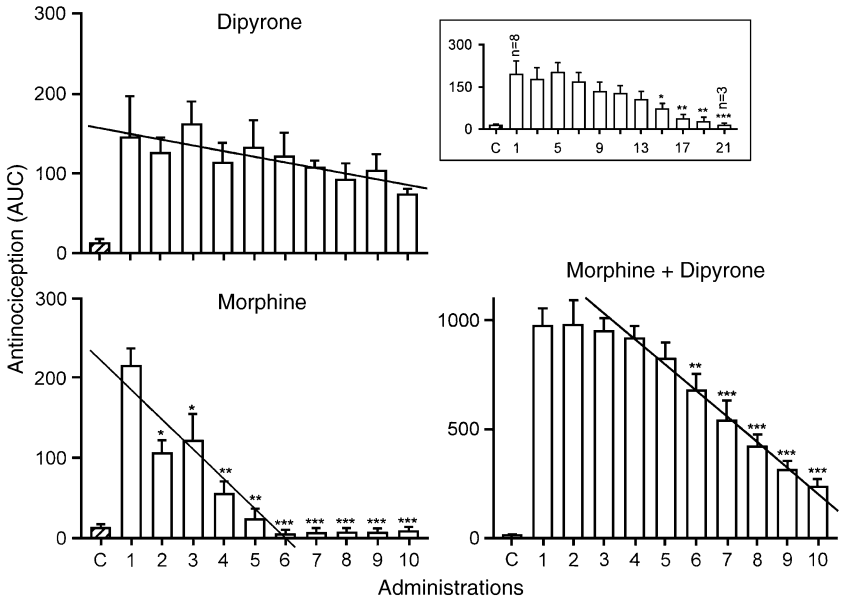

Fig. 3. Antinociceptive effects of 10 repeated administrations of 600 mg/kg dipyrone, 3.1 mg/kg morphine, or the combination of the same doses of morphine plus dipyrone measured as area under the curve (AUC) obtained from time course curves. Continuous lines represent the linear regressions used to determine trends in the development of tolerance. Insert shows the effects of 21 administrations of 600 mg/kg dipyrone. Data are expressed as mean ± S.E.M. of eight determinations. Experimental data were adjusted with the following equations: Dipyrone $y = -7.2x + 157$ ($r^2 = 0.71$); morphine $y = -39x + 224$ ($r^2 = 0.89$); combination of morphine plus dipyrone $y = -119x + 1389$ ($r^2 = 0.99$). Asterisks denote statistically significant differences from the effects observed with the first drug administration $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; Tukey test.

$P = 0.37$). In spite of this, the linear regression suggested a trend towards the development of tolerance to the antinociceptive effects of dipyrone (slope $= -7.2$; $P < 0.05$) and predicted that no effect would be observed at the 22nd administration (when $y = 0$, $x = 21.8$). The effects of morphine are also shown in this figure. The analysis of the area under the curve confirmed that repeated morphine administration resulted in a gradual development of tolerance that was complete after the fifth administration. This experimental finding closely resembles the time prediction given by linear regression to tolerance development (i.e., when $y = 0$, $x = 5.7$). The combination of morphine plus dipyrone produced similar antinociceptive effects at the first five administrations, but a gradual development of tolerance was observed from the sixth administration onwards (slope $= -119$; $P < 0.001$). In this case, the prediction by linear regression to develop tolerance was at the 12th administration (when $y = 0$, $x = 11.7$). Since the regression analysis predicted that complete tolerance would be evident at the 22nd administration of dipyrone, an additional group of rats was tested to assess the validity of this prediction. Animals received 600 mg/kg dipyrone twice a day, as described before, for 11 days. As previously mentioned, the latency to tail withdrawal was determined only after morning administrations. Results are shown in the insert of Fig. 3. As seen, complete tolerance was observed at the 19th administration, a finding reasonably close to the theoretical prediction. The number of animals diminished by the end of the study due to loss of cannulae.

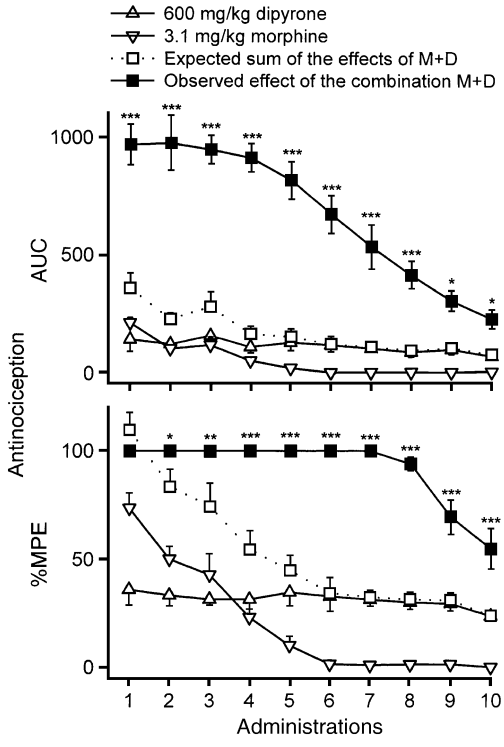

Fig. 4. Antinociceptive effects of repeated administration of dipyrone, morphine or the combination of the same doses of morphine plus dipyrone (M + D), expressed as the area under the curve (AUC, upper panel) and as the percent of maximum possible effect (%MPE, lower panel). Each point represents the mean ± S.E.M. of eight rats. Asterisks denote statistically significant differences from the expected sum of the effects of morphine plus dipyrone in each administration. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; Tukey test.

3.4. Antinociceptive potentiation

To further analyse the synergistic actions produced by the combination of morphine plus dipyrone, we calculated the expected theoretical sum of the antinociceptive effect produced by each individual analgesic compound and compared it with the experimentally antinociception observed with the combined treatment. The effects are expressed as the area under the curve and as the percentage of maximum possible effect and shown in Fig. 4. For comparative purposes, the antinociception values obtained with individual drugs previously described in Fig. 3 are also included in this figure. The combination morphine/dipyrone produced an antinociceptive effect consistently higher than the expected sum of the antinociceptive effects of individual agents (upper panel). This potentiation was more evident for the first five administrations, but remained statistically significant until the 10th administration ($F(1,140) = 379.8$, $P < 0.001$; two-way ANOVA). When data were expressed as percentage of maximum possible effect (lower panel), the potentiation appeared as less evident for the first three administrations than with the previous analysis. This apparent lower potentiation is in all probability due to the bias produced by the cut-off value.

4. Discussion

There is a consensus that non-steroidal anti-inflammatory drugs improve the antinociceptive effects of opioids when both types of drugs are co-administered acutely (Grotto et al., 1965; Malmberg and Yaksh, 1993; Sandrini et al., 1998; Maves et al., 1994; Christie et al., 1999; Lashbrook et al., 1999). In particular, it has been demonstrated that dipyrone produces an acute enhancement of morphine-induced antinociception in several animal models of nociception (Taylor et al., 1998; Aguirre-Bañuelos and Granados-Soto, 1999; Carlsson and Jurna, 1987; Lopez-Muñoz, 1994). The effectiveness of the combination of opioids with non-steroidal anti-inflammatory drugs has also been long recognised by clinicians (Calimlim et al., 1976; Bentley and Head, 1987; Picard et al., 1997) and the World Health Organization, who, since 1986, recommends the use of these combinations as the final step of the analgesic treatment ladder (World Health Organization, 1986). In spite of this, only very recently, the efficacy of combined analgesic drugs throughout chronic treatment has been tested in controlled laboratory studies. We have recently reported that the combined administration of morphine plus dipyrone produces an analgesic potentiation not only acutely, but also in arthritic rats treated once a day for 12 days with this combination. For methodological reasons, the effectiveness of the combination was tested only at the beginning and at the end of the study in independent groups of animals (Hernandez-Delgadillo et al., 2002). The purpose of the present work was to systematically evaluate the time course of the antinociceptive efficacy of the combined administration of morphine plus dipyrone along 10 repeated administrations in order to analyse if tolerance develops and potentiation persists with this treatment schedule.

The tail-flick test was selected to perform this study because it allows the repeated evaluation of antinociception in the same group of animals for relatively long periods of time (Nance and Sawynok, 1987). There is some controversy about the usefulness of the tail-flick test for the evaluation of the antinociceptive efficacy of non-steroidal anti-inflammatory drugs. Some authors consider that non-steroidal anti-inflammatory drugs are not effective (Akman et al., 1996; Bjorkman et al., 1990; Taylor et al., 1998; Powell et al., 1999; Wong et al., 2000). In contrast, other groups have found that dipyrone is effective against the nociception produced by radiant heat, at doses even lower than those used in this work (Carlsson et al., 1986; Carlsson and Jurna, 1987; Jones, 1996; Tortorici and Vanegas, 1994; Tortorici et al., 1996). The discrepancies in the literature are most probably due to species differences and to the intensity of the thermal stimulus applied (Hunskaar et al., 1986). In fact, in preliminary experiments in our lab, dipyrone lost its antinociceptive effects when the intensity of the thermal stimulus was increased (data not shown).

The antinociception produced by dipyrone was milder and had a longer onset of action than that produced by morphine alone or in combination with dipyrone. In addition, the antinociceptive effects of the combined administration of morphine and dipyrone lasted longer than the effects of each separate compound. Both the shorter onset of action and the increased duration of antinociception constitute beneficial aspects of the combination of morphine plus dipyrone.

The development of tolerance after repeated administration of opioids is a well-known undesired effect that limits their use for prolonged periods of time (Bhargava, 1994; Gutstein and Akil, 2001). In the present study, using a dose schedule of two administrations a day for 5 days, tolerance to morphine (3.1 mg/kg) began after the second administration and was complete after the fifth. In many studies involving chronic treatment with opioids, two closely related phenomena occur: the development of tolerance and the development of physical dependence. Interestingly, in our case the rapid development of tolerance to morphine-antinociceptive effects was not accompanied by an observable state of physical dependence. The occurrence of hyperalgesia (VonVoigtlander and Lewis, 1983; Tilson et al., 1973) and the precipitation of an abstinence response by the administration of an opioid antagonist (Francis and Schneider, 1971; Wei et al., 1973) are both considered reliable indexes of physical dependence in opioid-treated animals. No changes in the basal latency to tail withdrawal were seen before each opioid administration throughout the present study, indicating that hyperalgesia did not occur. Additionally, some animals were challenged with 3.1 mg/kg naloxone i.v. after the 10th administration of morphine and

the characteristic signs of abstinence were not seen (data not shown). Based on these findings, we suggest that the present protocol can be used to induce antinociceptive tolerance to morphine without developing an important state of physical dependence.

It is generally considered that non-steroidal anti-inflammatory drugs do not produce tolerance to their antinociceptive effects (see, for instance, Sunshine and Olson, 1994; Roberts and Morrow, 2001). In agreement with this notion, the initial 5-day treatment used in the present study did not result in clear tolerance. However, a trend analysis of the same data did suggest that such a phenomenon could occur with a more prolonged treatment. That this was indeed the case was confirmed by the results obtained with an 11-day treatment with dipyrone. There are technical difficulties to conduct chronic nociception studies. Among them, the most frequent are the limited number of times that an animal can be subjected to a nociceptive stimulus without causing tissue damage and the difficulty to maintain an i.v. cannula correctly fixed in its place for prolonged periods of time. These problems usually lead to a decrease in the number of animals available at the end of the study. Based on the good predictability observed in the present work for the development of tolerance to the different treatments followed, it seems that trend analysis could be a helpful approach to predict the tolerance potential of analgesic compounds whenever chronic studies are difficult to conduct.

In a recent report, Tortorici and Vanegas (2000) showed that repeated microinjection of dipyrone into the periaqueductal gray matter twice a day induces antinociceptive tolerance in rats after 2 days of treatment evaluated in the tail flick and the hot plate tests. Our results support these findings and show for the first time that tolerance to the antinociceptive effects of dipyrone can also occur with repeated systemic administration. It would be important to determine if tolerance to dipyrone develops against nociceptive stimuli other than thermal (mechanical, chemical, etc.).

To our knowledge, the time course of the development of tolerance with combined treatments of morphine and dipyrone has not been studied in controlled conditions in the lab. Our findings show that the efficacy of the combination of morphine plus dipyrone was relatively stable during the first administrations, but decreased towards the end of the treatment. It is of interest that tolerance developed more slowly to the combined treatment of morphine and dipyrone than to morphine alone. The time span of the experimental design in chronic studies seems to be crucial to reach a conclusion. For example, if our study would have been restricted to five administrations, we would have concluded that dipyrone prevented the development of tolerance to morphine; however, with a longer period of observation, it became evident that dipyrone delayed, rather than prevented, tolerance development. In this regard, there is recent evidence suggesting that tolerance to morphine can be prevented with the simultaneous i.t. administration of non-steroidal anti-inflammatory drugs (ketorolac, ibuprofen, or indomethacin) (Powell et al., 1999; Wong et al., 2000). Whether these compounds really differ from dipyrone in their effects when combined with opioids or the differences are due to the duration of the treatment would be the matter of further investigation.

As previously mentioned, the combined administration of morphine and dipyrone produced significantly greater effects than the additive actions of each drug given individually, suggesting that potentiation does occur (Seegers et al., 1981). The synergism observed in the present study was particularly evident at the beginning of the treatment and decreased after repeated administration. Since, by definition, the percentage of maximum possible effect strongly depends on the cut-off value, the best approach to study the potentiation of an analgesic combination seems to be the analysis of the area under the curve of the time courses of the antinociceptive effects.

At the present moment, we do not have an explanation concerning the mechanisms underlying the potentiation observed between morphine and dipyrone. A pharmacokinetic interaction between morphine and dipyrone cannot be discarded and further research on this topic is guaranteed.

Several reports indicate that dipyrone effects are due, at least in part, to a release of endogenous opioids in the descending pain pathways (Tortorici and Vanegas, 1994, 2000; Tortorici et al., 1996; Jones, 1996). Although this mechanism could explain the increased antinociceptive effect observed with the combined administration of morphine plus dipyrone, it would not explain the delay in tolerance development observed in our study. On the contrary, if the increased antinociceptive effect would be the result of an increase in opioid availability, one would expect tolerance to develop more rapidly. Another possibility is that some of the mechanisms that have been proposed to be effective in the prevention of tolerance development could play a role in the delay of tolerance development and/or the potentiation observed with the combination of morphine plus dipyrone. Among them, NMDA receptor antagonism (Trujillo and Akil, 1991, 1994; Marek et al., 1991; Tiseo and Inturrisi, 1993; Allen and Dykstra, 1999), and cyclooxygenase inhibition (Powell et al., 1999; Wong et al., 2000), are two well-characterised phenomena. There are several reports indicating that dipyrone acts as a nonspecific cyclooxygenase inhibitor (Weithmann and Alpermann, 1985; Campos et al., 1999), and causes significant inhibition of [^{3}H]glutamate binding in cerebral cortical membranes from both mice and rats (Beirith et al., 1998). Up to this moment, however, the mechanisms underlying antinociceptive potentiation and tolerance development retardation are not clear and deserve further investigation.

In conclusion, the combined administration of morphine plus dipyrone produced a potentiation of their individual antinociceptive effects that was higher at the beginning than at the end of the treatment. Tolerance to the antinociceptive effects of the combination morphine/dipyrone developed

more slowly than tolerance to morphine alone. These data provide pre-clinical support to the widespread use of combinations of non-steroidal anti-inflammatory drugs and opioids for the treatment of chronic pain.

Acknowledgements

This work was taken in part from G.P. Hernández-Delgadillo doctoral dissertation and was supported by grant 30571M (S.L.C.) and scholarship 125264 (G.P.H.) from Conacyt. Authors wish to thank Dr. Ricardo Plancarte for his continuous support to this work and Mr. Isaí Méndez and Abraham Contreras for their technical assistance.

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.

Akman, H., Aksu, F., Gultekin, I., Ozbek, H., Oral, U., Doran, F., Baysal, F., 1996. A possible central antinociceptive effect of dipyrone in mice. Pharmacology 53, 71–78.

Allen, R.M., Dykstra, L.A., 1999. The competitive NMDA antagonist LY235959 modulates the progression of morphine tolerance in rats. Psychopharmacology 142, 209–214.

Beirith, A., Santos, A.R.S., Rodrigues, A.L.S., Creczynski-Pasa, T.B., Calixto, B., 1998. Spinal and supraspinal antinociceptive action of dipyrone in formalin, capsaicin and glutamate tests. Study of the mechanism of action. Eur. J. Pharmacol. 345, 233–245.

Bentley, K.C., Head, T.W., 1987. The additive analgesic efficacy of acetaminophen, 1000 mg, and codeine, 60 mg, in dental pain. Clin. Pharmacol. Ther. 42, 634–640.

Bhargava, H.N., 1994. Diversity of agents that modify opioid tolerance, physical dependence, abstinence syndrome and self-administrative behavior. Pharmacol. Rev. 46, 293–324.

Bjorkman, R.L., Hedner, J., Hedner, T., Henning, M., 1990. Central, naloxone-reversible antinociception by diclofenac in the rat. Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 171–176.

Calimlim, J.F., Wardell, W.M., Davis, H.T., Lasagna, L., Gillies, A.J., 1976. Analgesic efficacy of an orally administered combination of pentazocine and aspirin. Clin. Pharmacol. Ther. 21, 34–43.

Campos, C., De Gregorio, R., García-Nieto, R., Gago, F., Ortiz, P., Alemany, S., 1999. Regulation of cyclooxygenase activity by metamizol. Eur. J. Pharmacol. 378, 339–347.

Carlsson, K.H., Jurna, I., 1987. The role of descending inhibition in the antinociceptive effects of the pyrazolone derivatives, metamizol (dipyrone) and aminophenazone (''Pyramidon''). Naunyn-Schmiedeberg's Arch. Pharmacol. 335, 154–159.

Carlsson, K.H., Helmreich, J., Jurna, I., 1986. Activation of inhibition from the periaqueductal grey matter mediates central analgesic effect of metamizol (dipyrone). Pain 27, 373–390.

Christie, M.J., Vaughan, C.W., Ingram, S.L., 1999. Opioids, NSAIDS and 5-lipoxygenase inhibitors act synergistically in brain via arachidonic acid metabolism. Inflammation Res. 48, 1–4.

Cox, L.S., Tiffany, S.T., 1997. Associative and nonassociative tolerance: the effects of dose and interdose interval. Pharmacol. Biochem. Behav. 57, 31–36.

D'Amour, F.E., Smith, D.L., 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72, 74–79.

Francis, D.L., Schneider, C., 1971. Jumping after naloxone precipitated withdrawal of chronic morphine in the rat. Br. J. Pharmacol. 41, 424P–425P.

Garcia-Alonso, F., Gonzalez de Suso, M.J., Lopez-Alvarez, M., Palop, R., 1991. Comparative study of the efficacy of dipyrone, diclofenac sodium and pethidine in acute renal colic. Eur. J. Clin. Pharmacol. 40, 543–546.

Gibaldi, M., 1991. Estimation of area under the curve. In: Gibaldi, M. (Ed.), Biopharmaceutics and Clinical Pharmacokinetics, 4th ed. Lea & Febiger, Pennsylvania, USA, pp. 377–378.

Grotto, M., Dikstein, S., Sulman, F.G., 1965. Additive and augmentative synergism between analgesic drugs. Arch. Int. Pharmacodyn. 155, 365–372.

Gutstein, H.B., Akil, H., 2001. Opioid analgesics. In: Hardman, J.G., Limbird, L.E., Goodman Gilman, A. (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed. McGraw-Hill, USA, pp. 569–619.

Hernandez-Delgadillo, G.P., Ventura, R., Diaz, M.I., Dominguez, A.M., Lopez-Muñoz, F.J., 2002. Metamizol potentiates morphine antinociception but not constipation after chronic treatment. Eur. J. Pharmacol. 441, 177–183.

Hunskaar, S., Berge, O.G., Hole, K., 1986. A modified hot-plate test sensitive to mild analgesics. Behav. Brain Res. 21, 101–108.

Jones, S.L., 1996. Dipyrone into the nucleus raphe magnus inhibits the rat nociceptive tail-flick reflex. Eur. J. Pharmacol. 318, 37–40.

Lasagna, L., 1965. Drug interaction in the field of analgesic drugs. Proc. R. Soc. Med. 58, 978–983.

Lashbrook, J.M., Ossipov, M.H., Hunter, J.C., Raffa, R.B., Tallarida, R.J., Porreca, F., 1999. Synergistic antiallodynic effects of spinal morphine with ketorolac and selective COX_1- and COX_2-inhibitors in nerve-injured rats. Pain 82, 65–72.

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

Malmberg, A.B., Yaksh, T.L., 1993. Pharmacology of the spinal action of ketorolac, morphine, ST-91, U50488H, and L-PIA on the formalin test and an isobolographic analysis of the NSAID interaction. Anesthesiology 79, 270–281.

Marek, P., Ben-Eliyahu, S., Gold, M., Liebeskind, J.C., 1991. Excitatory amino acid antagonists (kynurenic acid and MK-801) attenuate the development of morphine tolerance in the rat. Brain Res. 547, 77–81.

Martin, T.J., Eisenach, J.C., 2001. Pharmacology of opioid and nonopioid analgesics in chronic pain states. J. Pharmacol. Exp. Ther. 299, 811–817.

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, 1094–1101.

Mercadante, S., 1999. Problems of long-term spinal opioid treatment in advanced cancer patients. Pain 79, 1–13.

Miralles, R., Cami, J., Gutierrez, J., Torne, J., Garces, J.M., Badenas, J.M., 1987. Diclofenac versus dipyrone in acute renal colic: a double-blind controlled trial. Eur. J. Clin. Pharmacol. 33, 527–528.

Nance, P.W., Sawynok, J., 1987. Substance P-induced long-term blockade of spinal adrenergic analgesia: reversal by morphine and naloxone. J. Pharmacol. Exp. Ther. 240, 972–977.

Patel, C.V., Kappikar, M.G., Patel, M.S., Parulkar, G.B., Pinto, P.L.M., 1980. Management of pain after abdominal surgery: dipyrone compared with pethidine. Br. J. Clin. Pharmacol. 10, 351S–354S.

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

Planas, M.E., Gay-Escoda, C., Bagan, J.V., Santamaria, J., Peñarrocha, M., Donado, M., Puerta, J.L., Garcia-Magaz, I., Ruiz, J., Ortiz, P., 1998. Oral metamizol (1 g and 2 g) versus ibuprofen and placebo in the treatment of lower third molar surgery pain: randomized double-blind multi-centre study. Eur. J. Clin. Pharmacol. 53, 405–409.

Powell, K.J., Hosokawa, A., Bell, A., Sutak, M., Milne, B., Quirion, R., Jhamandas, K., 1999. Comparative effects of cyclo-oxygenase and nitric oxide synthase inhibition on the development and reversal of spinal opioid tolerance. Br. J. Pharmacol. 127, 631–644.

Roberts, L.J., Morrow, J.D., 2001. Analgesic-antipyretic and antiinflamma-

tory agents and drugs employed in the treatment of gout. In: Hardman, J.G., Limbird, L.E., Goodman Gilman, A. (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed. McGraw-Hill, USA, pp. 687–731.

Rodriguez, M., Barutell, C., Rull, M., Galvez, R., Pallares, J., Vidal, F., Aliaga, L., Moreno, J., Puerta, J., Ortiz, P., 1994. Efficacy and tolerance of oral dipyrone versus oral morphine for cancer pain. Eur. J. Cancer 30A, 584–587.

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, 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. Inflammation Res. 48, 120–127.

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

Sunshine, A., Olson, N.Z., 1994. Nonnarcotic analgesics. In: Wall, P.D., Melzack, R. (Eds.), Textbook of Pain, 3rd ed. Churchill Livingstone, USA, pp. 923–942.

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.

Tilson, H.A., Rech, R.H., Stolman, S., 1973. Hyperalgesia during withdrawal as a means of measuring the degree of dependence in morphine dependent rats. Psychopharmacologia 28, 287–300.

Tiseo, P.J., Inturrisi, C.E., 1993. Attenuation and reversal of morphine tolerance by the competitive *N*-methyl-D-aspartate receptor antagonist, LY274614. J. Pharmacol. Exp. Ther. 264, 1090–1096.

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

Tortorici, V., Vanegas, H., 2000. Opioid tolerance induced by metamizol (dipyrone) microinjections into the periaqueductal grey of rats. Eur. J. Neurosci. 12, 4074–4080.

Tortorici, V., Vasquez, E., Vanegas, H., 1996. 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. 725, 106–110.

Trujillo, K.A., Akil, H., 1991. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 251, 85–87.

Trujillo, K.A., Akil, H., 1994. Inhibition of opiate tolerance by non-competitive *N*-methyl-D-aspartate receptor antagonists. Brain Res. 633, 178–188.

VonVoigtlander, P.F., Lewis, R.A., 1983. A withdrawal hyperalgesia test for physical dependence: evaluation of mu and mixed-partial opioid agonists. J. Pharmacol. Methods 10, 277–282.

Wei, E., Loh, H.H., Way, E.L., 1973. Quantitative aspects of precipitated abstinence in morphine-dependent rats. J. Pharmacol. Exp. Ther. 184, 398–403.

Weithmann, K.U., Alpermann, H.G., 1985. Biochemical and pharmacological effects of dipyrone and its metabolites in model systems related to arachidonic acid cascade. Arzeinmittel.-Forsch. 35, 947–952.

Wong, C.S., Hsu, M.M., Chou, R., Chou, Y.Y., Tung, C.S., 2000. Intrathecal cyclooxygenase inhibitor administration attenuates morphine antinociceptive tolerance in rats. Br. J. Anaesth. 85, 747–751.

World Health Organization, 1986. Cancer Pain Relief. WHO Office of Publication, Geneva.

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