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D-propoxyphene and dipyrone co-administration produces greater antinociception and fewer adverse effects than single treatments in rats

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ABSTRACT

D-propoxyphene is a commonly prescribed opiate analgesic. Its use is limited by unwanted side effects at high doses and tolerance development after chronic administration. Dipyrone (also known as metamizol) is a non-steroidal anti-inflammatory drug extensively used in Latin America and Europe. The objective of this work was to evaluate the antinociceptive efficacy of a dipyrone/D-propoxyphene combination and the development of tolerance to its repeated administration in the tail flick test in rats. Male Wistar rats (200 ± 20 g) were i.v. injected twice daily (8 h apart) with 0.31 mg/kg D-propoxyphene, 400 mg/kg dipyrone, or the combination of these drugs, at the same doses, until complete tolerance was observed. A time course of the effects for each administration was determined. At the doses tested, D-propoxyphene and dipyrone produced mild antinociception per se. Repeated administration resulted in complete tolerance to their antinociceptive effects by the sixth dose. The D-propoxyphene/dipyrone combination produced more antinociception than expected by the sum of individual drug effects. With this treatment, tolerance developed at the 15th administration. In animals already tolerant to D-propoxyphene or dipyrone alone, subsequent administration of the combination partially restored the antinociceptive effect. These results suggest that the use of this combination provides advantages over single drug therapies.

1. Introduction

D-propoxyphene is a weak opiate analgesic drug widely prescribed throughout the world for the relief of mild to moderate pain (Mercadante et al., 1998; Collins et al., 2000; Goldstein and Turk, 2005). It has been used to ease withdrawal symptoms in opioid addicts (Inaba et al., 1974), and is now prescribed for pain treatment in elderly patients (Goldstein and Turk, 2005) and used for postoperative pain management. D-propoxyphene is an open chain mu opioid receptor agonist which also acts as a non-competitive N-methyl-D-aspartate receptor antagonist (Ebert et al., 1998; Miller, 1977; Soni and Van Gelder, 1981). As it occurs with several opiates, chronic D-propoxyphene administration is limited by tolerance development to its analgesic effects which can lead to a gradual dose escalation and an increase in the occurrence of undesirable effects. Among them, D-propoxyphene can produce somnolence, nausea and vomiting (Gustein and Akil, 2005; Barkin et al., 2006) and, at high doses, bradychardia and electrocardiogram alterations (Barkin et al., 2006).

A useful strategy to reduce unwanted opiate side effects is to combine low quantities of these drugs with low doses of non-steroidal anti-inflammatory compounds. In this regard, extensive preclinical and clinical research has shown that these combinations cannot only reduce adverse effects, but they can also produce analgesic potentiation (Sandrini et al., 1998; Lashbrook et al., 1999) and a delay in tolerance development (Hernández-Delgadillo et al., 2003).

Dipyrone (also known as metamizol) is a non-steroidal anti-inflammatory drug widely used in Latin America, Germany and several European countries (Arcila-Herrera et al., 2004). Some preclinical reports have shown that acutely, dipyrone enhances morphine-induced antinociception (Carlsson and Jurna, 1987; López-Muñoz et al., 1994). In a recent work using an inflammatory nociception test, our group reported that a morphine/dipyrone combination produces antinociceptive potentiation not only in acutely-treated rats, but also in animals already tolerant to each individual drug (Hernández-Delgadillo and Cruz, 2004). Moreover, this potentiation occurs without an increase in constipation (Hernández-Delgadillo et al., 2003).

Based on these data, the objective of the present work was to study the time course of tolerance development and antinociceptive potentiation throughout repeated administration of a combined treatment of D-propoxyphene and dipyrone in the tail-flick test in rats. 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

Adult male Wistar rats (180–220 g) from our own breeding facilities were used in this study. Rats were housed in an animal room

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with controlled temperature (22 ± 2 °C) under a 12:12-h light–dark cycle with free access to drinking water and commercial food. In order to reduce stress, all animals were handled twice daily for two days before drug testing. All experimental procedures followed the regulations established in the Mexican official norm for the use and care of laboratory animals "NOM-062-ZOO-1999" and the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983).

2.2. Drugs

D-propoxyphene chloride was obtained from the Mexican Health Secretariat. Dipyrone sodium was purchased from Aventis (Mexico City, Mexico), heparin sodium, from Sigma (St. Louis, MO, USA), ketamine hydrochloride, from Probiomed (Mexico City, Mexico) and xylazine hydrochloride, from Pisa (Tula Hidalgo, Mexico).

2.3. Surgical procedure and drug administration

In order to place the cannula, rats were briefly anaesthetised with a. ketamine/xylazine mixture (45/12 mg/kg, i.p.). A polyethylene catheter (PE50), flushed with heparin solution, 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 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. All drugs were dissolved in sterile saline solution and administered intravenously in a final volume of 1 ml during 2 min using an infusion pump (KD Scientific, USA). For the combination, D-propoxyphene was dissolved in saline solution and then mixed with dipyrone to be administered in a single 1-ml infusion. After each drug injection, the catheter was flushed with heparin solution in a volume exceeding the estimated catheter dead space.

2.4. Antinociceptive activity evaluation

A standardised tail-flick apparatus, with a radiant heat source connected to an automatic timer (UGO BASILE, Italy) was used for nociception assessment. In this model, antinociception is seen as an increase in tail withdrawal latency (D'Amour and Smith, 1941; modified by Nance and Sawynok, 1987). At the beginning of the study, the stimulus intensity was adjusted to get a baseline tail-flick latency of 6.0 ± 0.5 s. Animals were screened for thermal nociception before the first drug injection; rats showing no flicking within 5.5 to 6.5 (10–15% of the total) were discarded. 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 three tests was obtained. After drug administration, tail withdrawal latency was determined every 15 min for the first hour, and every 30 min for the subsequent hour. Rats were euthanised with carbon dioxide at the end of the experiments.

2.5. Study design

2.5.1. Protocol 1: dose–response curves

Independent groups of rats ($n = 6$, each) were used to complete dose–response curves for D-propoxyphene (0.31 to 31.6 mg/kg) and dipyrone (100, 400 and 600 mg/kg) in order to select the doses to be used in chronic combination studies.

2.5.2. Protocol 2: potentiation and tolerance development

Six independent groups of rats were used ($n = 6$, each). Four of them received D-propoxyphene (0.31 or 10 mg/kg) or dipyrone (400 or 600 mg/kg). Each drug was administered at 8:00 and 16:00 h, for several days until antinociception disappeared. The lower drug doses (0.31 mg/kg D-propoxyphene and 400 mg/kg dipyrone) were selected because they were close to the calculated ED_{33} of each compound, and were suitable for combination studies. The higher doses were chosen based on their ability to produce maximal antinociception without the induction of limiting unwanted side effects. Another group of rats received 0.31 mg/kg D-propoxyphene plus 400 mg/kg dipyrone using the same administration schedule. Finally, control animals were injected with saline solution twice daily for up to five days. Although drugs were administered at 8:00 and 16:00 h, nociception was evaluated only after the morning dose to minimise the exposure to noxious stimulus in chronically-treated rats.

2.5.3. Protocol 3: effects of the antinociceptive combination in tolerant rats

Two groups of animals ($n = 6$) were administered twice daily with 0.31 mg/kg D-propoxyphene or 400 mg/kg dipyrone until no significant effect was observed (at the sixth administration). Eight hours later, both groups were switched to the combination of 0.31 mg/kg D-propoxyphene with 400 mg/kg dipyrone for five additional administrations. As in the previous experiment, injections were given twice a day, but nociception was evaluated only after the morning dose.

2.6. Data and statistical analysis

Results are expressed as the mean ± S.E.M. of six determinations. Antinociception was evaluated by: 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, which reflects the global antinociceptive effect along time, was calculated by the trapezoidal rule (Gibaldi, 1991). Comparisons between two experimental groups were done by Student's t test. This test was also used to compare the effect of D-propoxyphene plus dipyrone with the theoretical sum of their individual effects. The theoretical sum was calculated by adding the effects of each drug, either expressed as area under the curve after subtracting control values, or as the percentage of maximal possible effects. Comparisons among several drug treatments were done by a one-way analysis of variance followed by a Tukey test. To evaluate trends in tolerance development, a linear regression was used. The program used for Statistics was Sigma-Stat (version 2.03, Jandel Scientific). Dose–response curves were adjusted with the Win-nonlin program (Pharsight, version 2.1).

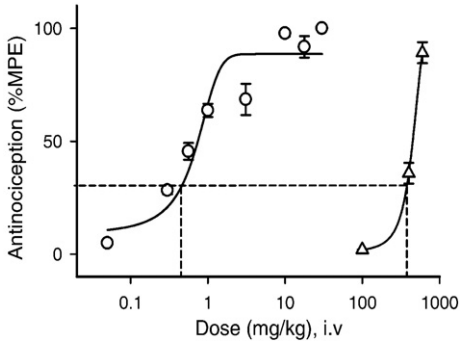

Fig. 1. Dose–response curves for D-propoxyphene (circles) and dipyrone (triangles) in the tail-flick test in rats. The parameter evaluated was the latency to tail-flick withdrawal as a function of dose. Antinociception is expressed as the percentage of maximum possible effect (%MEP). Each point represents the mean ± S.E.M. of six data points. Dashed lines represent the approximated ED_{33} of each drug.