Analysis of the opioid–opioid combinations according to the nociceptive stimulus in mice

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ARTICLE INFO

ABSTRACT

The purpose of the present study was to characterize the antinociceptive effects of tramadol, fentanyl and morphine, when two of them were systemically combined in a 1:1 potency ratio, in the hot plate, the acetic acid writhing, and the formalin tests in mice. Interaction indexes and isobolographic analysis were used to assess the type of interaction. Fentanyl was the most potent drug, followed by morphine and tramadol, with the exception in the phase I of formalin test. Synergistic interactions were obtained when tramadol was combined with fentanyl or with morphine in the writhing and formalin tests. But, in the hot plate only additive interactions were obtained. Changes were induced on the type of interaction depending on the level of effect of opioid–opioid combinations. Moreover, co-administration of fentanyl with morphine showed additivity, regardless of the type of stimulus. Standard rotarod test analysis confirmed intact motor coordination. The present findings suggest that the type of interaction between opioids is not only related to the nature of nociceptive stimulus but also to non-opioid analgesic pathways.

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1. Introduction

In pain management, not enough analgesia is achieved using monotherapy. The administration of two or more drugs (multimodal analgesia) is a widely used strategy to improve the analgesic efficacy and to reduce adverse side effects of drugs. Although the usefulness of the co-administration of drugs of the same pharmacological group is controversial, it is effective in some pathologies such as depression [1] and epilepsy [2]. In humans, opioids are frequently used for the relief of moderate to severe pain of different etiology, and empirically combined, without knowing if they interact. Some clinical evidences in cancer patients suggest that the combination of two opioids (morphine plus oxycodone, morphine plus fentanyl or methadone) can be a useful alternative to opioid monotherapy [3,4]. In addition, clinical data in the management of moderate to severe pain in postoperative patients, show that morphine combined with tramadol improves analgesia and decreases morphine requirements after abdominal surgery compared with morphine alone [5]. Similarly, the addition of intrathecal morphine to spinal fentanyl plus bupivacaine, significantly reduced persistent pain and prolonged the time to analgesic request [6]. However, Friedman et al. [7] did not find benefits when combining morphine and fentanyl administered by intravenous PCA after bowel surgery and Marcou et al. [8] described the presence of antagonism between morphine and tramadol.

Thus, the benefits of two or more drugs simultaneously administered, should be evaluated before the combination can be considered useful. The pharmacological effects (beneficial and/or adverse) attained using combination treatment can be studied and additive, or non-additive effects (synergy or antagonism interaction) between drugs can occur [9].

Studies in rodents using different nociceptive assays have reported synergistic pain relief (supraadditive antinociceptive effects) following simultaneous administration of opioid agonists with different selectivity for μ-, δ and κ-OR at spinal site [10], or combining supraspinal and spinal sites [11,12], spinal and systemic routes [13–15]. Hence, synergy is usual in opioid pharmacology, but not all μ opioid agonists in combination interact [16]. Studies in animal models have reported that drug–drug interactions can be altered by different factors such as the ratio of the combinations [17], the presence of inflammation or morphine tolerance [18]. Moreover, Loomis et al. [19] described that the nature of the nociceptive stimulus evaluated could change the type of interaction between opioids co-administered in rats. All these results indicate a complex analgesic interaction between opioids.

The present study reports the analysis of the interaction when two opioids such as morphine (standard reference drug), fentanyl or tramadol are combined in mice. These drug combinations are often used in clinical practise in different situations. For example,

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cancer pain patients often receive chronic slow release morphine and oral transmucosal fentanyl for breakthrough pain [20,21]. In the management of acute postoperative pain tramadol is frequently used as analgesic, and morphine or fentanyl administered as rescue medication [22–24]. Thus, the drugs used in our study were selected on the basis of their clinical use. However, these and other opioid–opioid combinations are used empirically in humans, without knowing if the drugs interact with each other.

It has been found that synergy (when it happens) is a function of the proportions in the combination [9], and the fixed-ratio design was used in this study. In this protocol, the drugs are administered in amounts (doses) that keep the proportions of each constant. Although the drugs studied induce their antinociceptive effects by opioidergic mechanisms mainly, other possible pathways could be implicated. Then, the analgesic effects could change not only with the drug pair and the ratio of the two components, but also according to the level of effect, a fact that may be relevant when attempting to introduce drug combinations in clinical practise. Moreover, we also studied if the nature of noxious stimulus could modify the type of interaction between those opioids combined.

2. Material and methods

2.1. Animals

Male CD1 mice, weighting 25–30 g (Charles River, France) were used in this study. The experiments were performed according to the Ethical Guidelines of the International Association for the Study of Pain and the Ethical Committee for Animal Welfare of the Institution approved the protocols. Mice were housed in plastic cages (five mice per cage) with soft bedding and free access to food and water. They were maintained in a controlled temperature ($22 \pm 1\,^\circ$C and 60% relative humidity) and light (12:12 dark:light cycle with light on at 8:00) environment. Behavioural testing was performed between 09:00 a.m. and 17:00 p.m., in a quiet room. Mice were used only once and were killed at the end of the experiment by cervical dislocation.

2.2. Drugs

We used the same commercial drugs administered to humans in the clinical practise. Drugs were obtained from the following sources: tramadol (TRM) (Grünenthal, Madrid, Spain); fentanyl (FEN) (Kern Pharma, Barcelona, Spain) and morphine hydrochloride (M) (Alcaliber, Madrid, Spain). Individual drugs and their combinations were dissolved in saline solution (0.9%) just before use, and the drugs were administered subcutaneously (s.c.) at the nape of the neck in a volume of 0.250 ml, 30 min before behavioural testing, on the basis of previous reports [25,26].

2.3. Nociceptive tests

Each drug was administered at the following doses: TRM (1, 3, 5, 10, 30 and 50 mg/kg in the hot plate; 3,7, 8, 10 and 30 mg/kg in the writhing test and 1, 3,10, 30 and 100 mg/kg in both phases of the formalin test), FEN (0.01, 0.02, 0.03, 0.04, 0.05 and 0.07 mg/kg in each of the tests) and M (0.5, 1, 2, 3, 4 and 7 mg/kg in the hot plate; 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/kg in the writhing and 0.05, 0.1, 0.3, 1 and 3 mg/kg in the phase I and 0.3, 1, 3, 7 and 10 mg/kg in the phase II of the formalin test).

2.3.1. Formalin test

The method described by Rosland et al. [27] was used. To carry out the test 20 μl of a 5% formalin solution, was injected into the dorsal surface of the mice right hind paw, with a 27-gauge needle attached to a 50 μl Hamilton syringe. Each mouse was immediately returned to a plexiglass observation cylinder especially designed. The degree of pain intensity was recorded as the total time spent by the animal licking the injected paw, measured by visual observation and a digital time-out stopwatch. The test shows two clear cut phases: *Phase I* corresponds to the 5 min period starting immediately after the formalin injection. This initial phase represents a tonic acute pain due to peripheral nociceptor sensitization. *Phase II* was recorded as the 10 min period starting 20 min after the formalin administration and corresponds to inflammatory pain. The time of both phases was not registered due to that corresponds to a period of stillness or of not-activity. Control animals ($n = 34$) were injected with saline. For each drug, analgesic effects were characterized after the administration of a minimum of five doses. The licking times observed were converted to % maximum possible effect (MPE) as follows:

$$\%MPE = \left[\frac{\text{control licking time} - \text{postdrug licking time}}{\text{control licking time}} \right] \times 100$$

2.3.2. Writhing test

The procedure used has been described previously [28]. Briefly, mice were injected intraperitoneally (i.p.) with 10 ml/kg of 0.6% acetic acid solution, 30 min after the subcutaneous (s.c.) administration of the drugs, time at which preliminary experiments showed occurrence of the maximum effect of all drugs used. Each mouse was then placed in an individual clear plexiglass observation cylinder. A writhe is characterized by a wave of contraction of the abdominal musculature, accompanied by the elongation of the body and extension of one or both hind limbs. The number of writhes in a 5 min period was counted, starting 5 min after the acetic acid administration. Antinociception was expressed as percent inhibition of the number of writhes observed in control animals ($n = 23$). The results are expressed as maximum possible effect (%MPE) according to the following expression:

$$\%MPE = \left[\frac{\text{writhes in control mice} - \text{writhes postdrug mice}}{\text{writhes in control mice}} \right] \times 100$$

2.3.3. Hot plate test

The hot plate test was performed using an electronically controlled hot plate analgesia meter (Columbus Instruments, Columbus, OH, USA) heated to $52 \pm 1\,^\circ$C, described by Castañé et al. [29]. The nociceptive threshold evaluated was the time of the jumping response as the latency period. In absence of jumps, a 240 s cut-off was used to prevent tissue damage. Control animals ($n = 37$) were injected with saline. Percent analgesia was calculated as

$$\%MPE = \left[\frac{\text{latency postdrug} - \text{control latency}}{240 - \text{control latency}} \right] \times 100$$

2.4. Determination of motor functions: rotarod test

The rotarod test [30] was used to evaluate the effects of each opioid, individually (ED_{80}'s) and combined in 1:1 proportion, on motor coordination behaviour (LSI-Letica Scientific Instruments, Barcelona, Spain). We tested the highest values of the ED_{80}'s obtained for each individual opioid (in the different nociceptive tests). The doses used for drug combinations were those that induced synergistic antinociceptive effect at 80% of effect, to discriminate a possible synergism in impairment to motor function. The drugs were administered s.c. (at the nape of the neck), in a volume of 0.250 ml, 30 min before behavioural testing. Initially, all animals were trained to run on the rotarod apparatus on day 1, at a constant 10 rpm. Those mice that were unable to remain on the rod for two consecutive periods of 240 s (cut-off) were discarded. After a baseline trial of 240 s, the effects of the different drugs were