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SYNERGISM BETWEEN MORPHINE WITH FENTANYL IN EXPERIMENTAL MURINE ASSAYS

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ABSTRACT

Opioids are among the most effective pain relievers available; however induced antinociception has not been extensively studied in different animal pain models. The studies have been conducted with isolated opioids only, but have not been used in combination, as multimodal analgesia. In the present study, the pharmacological interaction of morphine with fentanyl was evaluated in different murine pain models by means of isobolographic analysis. In control animals, morphine and fentanyl produced a dose-related antinociceptive action in the murine assays and comparing the rank of potency was formalin hind paw phase I > formalin phase II > tail flick. The coadministration of morphine with fentanyl, in a fixed relation 1:1 of their ED_{50}, produces a synergistic interaction of different magnitude. The study shows that fentanyl is more effective than morphine. This disparity could be explained according to the suggestions that opioids could be acting through other targets either by different binding capacity, by the regulation or activation of non-opioid receptors. Furthermore, coadministration of morphine with fentanyl induces synergism in the murine trials, confirming the antinociceptive and anti-inflammatory capacity of the opioids.

KEYWORDS: Fentanyl, morphine, antinociception, anti-inflammation, synergism

INTRODUCTION

In the therapy of pain, opioids are among the most effective pain relievers available. Furthermore, these drugs have demonstrated analgesic efficacy in several animal models of pain. These tests include assays of tonic pain, as acetic acid writhing and formalin and assays of phasic pain as the tail flick and the hot plate. All of them can measure analgesia and anti-inflammation (Yam et al. 2020).

Opioids comprise a variety of drugs both natural (morphine, codeine) as synthetic (fentanyl, methadone), all effective in relief pain, but with potential capacity for induce dependence and abuse. Opioids activate specific trans-membrane G protein-coupled receptors known as mu-opioid (MOR), kappa-opioid (KOR), delta-opioid (DOR) and nociceptin-peptide-opioid (NOP) receptors, which are predominantly in the CNS and also in the SNP (Olson et al. 2019).

Activation of opioid receptors induces, among other signals, inhibition of adenylate cyclase, decreases opening calcium channels, increases potassium currents and activation of protein kinase C. These intracellular actions lead to decrease cellular excitability and consequently the neurotransmission. Opioids have a variety of effects, including pain relief, euphoria, drowsiness, sedation, constipation, nausea, vomiting, dizziness, depression respiratory, tolerance, physical dependence and addiction (Rosenblum et al. 2008; Corder et al. 2018).

Opioids can be classified on basis of their chemical structure as (1) opium alkaloids or opiates: codeine, morphine; (2) semisynthetic

derivatives of the natural alkaloids: oxycodone, hydrocodone, buprenorphine; (3) synthetic opioids: fentanyl, alfentanil, propoxyphene, methadone, loperamide, tramadol, pentadol (Patham and Williams, 2012). From the different opioids, due to their frequent use, they deserve to be highlighted: (A) morphine, a natural opioid, with potent analgesic activity by activation of MOR receptors in CNS and ¨PNS; (B) Fentanyl, a synthetic opioid 50 to 100 times more potent than morphine, is agonist at MOR receptors and effective in anaesthesia as well as maintenance (Xin et al. 2022).

There is a method of combining analgesic drugs, each with a different mechanism of action, called multimodal analgesia. This approach may be used in the treating of pain with opioids for reduction of undesirable effects (nausea, vomiting, respiratory depression, etc). When two drugs are taken together if the response result greater than the sum of individual response, the interaction is synergistic (Tallarida, 2001; Erik et al. 2017).

In the present study, the pharmacological cointeraction of morphine with fentanyl was evaluated in different murine pain models by means of isobolographic analysis.

MATERIALS AND METHODS

Animals

Male CF-1 mice (25-30g) housed on a 12h light-dark cycle at 22±1 °C with free access to food and water ad libitum, were used. All animal procedures were approved by the Animal Care and Use Committee at the Faculty of Medicine, University of Chile (Protocol CBA 0852/FMUCH/2018). Animals were acclimatized to the laboratory for at least 1h before testing, used only once during the protocol, and euthanized immediately after the algesimeter test by one intraperitoneal (i.p.) injection of 60mg/kg of pentobarbital. The number of animals was kept at a minimum, compatible with consistent effects of the drug treatment.

Measurement of antinociceptive activity

Antinociception was assessed by the following murine tests:

(A) tail-flick (TF) as described previously (Miranda et al. 2007). A radiant heat, automatic tail flick (Ugo Basile, Comerio, Italy) was used to measure response latencies. Baseline was obtained for all mice before protocol and then test latency measured after experimental administration of drugs. A cut-off time of 8 sec was set to avoid damage of mice tissue. Tail flick latencies controls were 2.65 ± 0.12 (n=12) and converted to % MPE.

(B) the formalin hind paw (FHP) test described previously was used (Miranda et al.,2007). To perform the test 20 µL of 2 % formalin solution was injected into the dorsal surface of the right hind paw. The intensity of pain was assessed as the total time, in sec, by the licking or biting of the injected paw. The test shows 2 clear cut-periods, each associated to a different type of pain. Phase I (THP-I) corresponding to the 5 min immediately after formalin injection and reflects tonic acute pain and phase II (THP-II), spans 10min, starting 20 min after formalin injection and reflects inflammatory pain. The control values were, phase I: 133.05 ± 7.04 (n =12) and phase II: 157.83 ± 9.10 (n=12). Licking time was converted to % MPE.

Experimental Design

In order to determine the antinociceptive potency of morphine (0.03, 0.06, 0.12 and 0.24 mg/kg i.p) and fentanyl (0.1, 0.3, 1 and 3 mg/kg, i.p.), a dose-response curve was obtained in the TF and FHP testsofmice using a least 6 animals for each 4 doses, as can be seen in figure 1. Then the DE_{50}, dose that induce 50% of MPE, was calculated from lineal regression of dose-response curves of morphine and fentanyl.

Isobolographic analysis

The method of isobolographic analysis was used to evaluate the interaction between morphine and fentanyl with the method previously described (Miranda et al. 2001). In summary, the isobolograms were constructed connecting ED_{50} of fentanyl in the abscissa with the ED_{50} of morphine in the ordinate to obtain the additive line. For each opioid combination, the experimental ED_{50} was obtained which was compared with the ED_{50} attained theoretically and denoted by a point on the additive line. A synergistic or supraadditive effect is considered

when the ED_{50} experimental is significantly lower than the theoretical ED_{50} and is represented by a point below the additive line. In addition, the nature and magnitude of the interaction of the combination is represented by the interaction index (I.I.) which is the ratio of combination potency, calculated as: I.I. = Experimental ED_{50} / theoretical ED_{50}. If the value is below 1, the interaction is supraadditive or synergistic.

Drugs

Drugs were freshly dissolved in sterile physiological salt solution of 10?mL/Kg, for intraperitoneal administration. Morphine hydrochloride and fentanyl hydrochloride were purchased from Sigma-Aldrich Chemical Co, St. Louis, Mo, USA.

Statistical Analysis

Results are presented as means ± SEM or 95 % confidence limits (95 % CL). The statistical difference between the results were assessed by one-way ANOVA, followed by Tukey's post-test for and p values less than 0.05 ($p<0.05$) were considered statistically significant. Statistical analyses were carried out using the program Pharm Tools Pro, version 1.27, Mc Cary Group Inc., PA, USA.

RESULTS

Antinociception of morphine and fentanyl

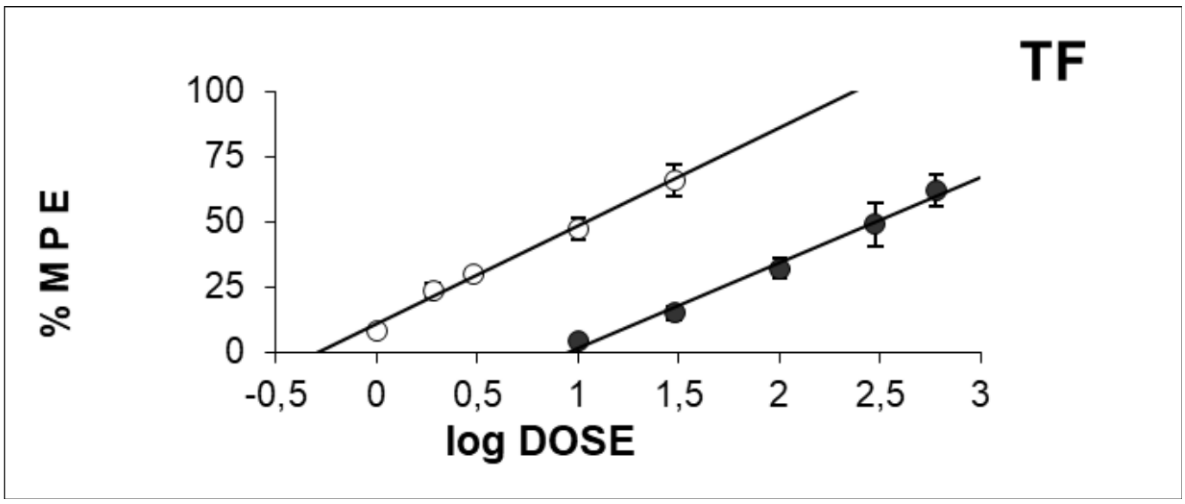
In control animals, the i.p. administration of morphine and fentanyl produced a dose-related antinociceptive action in the assays of mice, as can be seen in Figure 1.

TABLE 1. ED_{50} values wit SEM in mg/kg and analgesic ratio (AR) for the antinociceptive activity of morphine and fentanyl in algesimeter test of mice.

TEST	ED_{50} ± SEM MORPHINE	FENTANYL	AR^a	AR^b	AR^c
FHP-I	0.17 ± 0.02	0.055 ± 0.003	29.47	1.25	3.09
FHP-II	0.38 ± 0.03	0.033 ± 0.001	13.18	2.09	11.52
TF	5.01 ± 0.91	0.069 ± 0.002	1.00	1.00	72.60

FHP-I: formalin hind paw phase I, FHP-II: formalin hind paw phase II, TF: tail flick[a] compared with morphine TF, [b] compared with fentanyl TF, [c] comparison between morphine with fentanyl.

The corresponding ED_{50} with their respective SEM resultant from each assay is presented in Table 1. The order of analgesic ratio of morphine was: FHP-I > FHP-II > TF. In fentanyl the order of analgesic ratio was: FHP-II > FHP-I > TF. For the NSAIDs, the rank order of potency was: FHP-I > FHP-II > TF. All data are shown in Table 1.



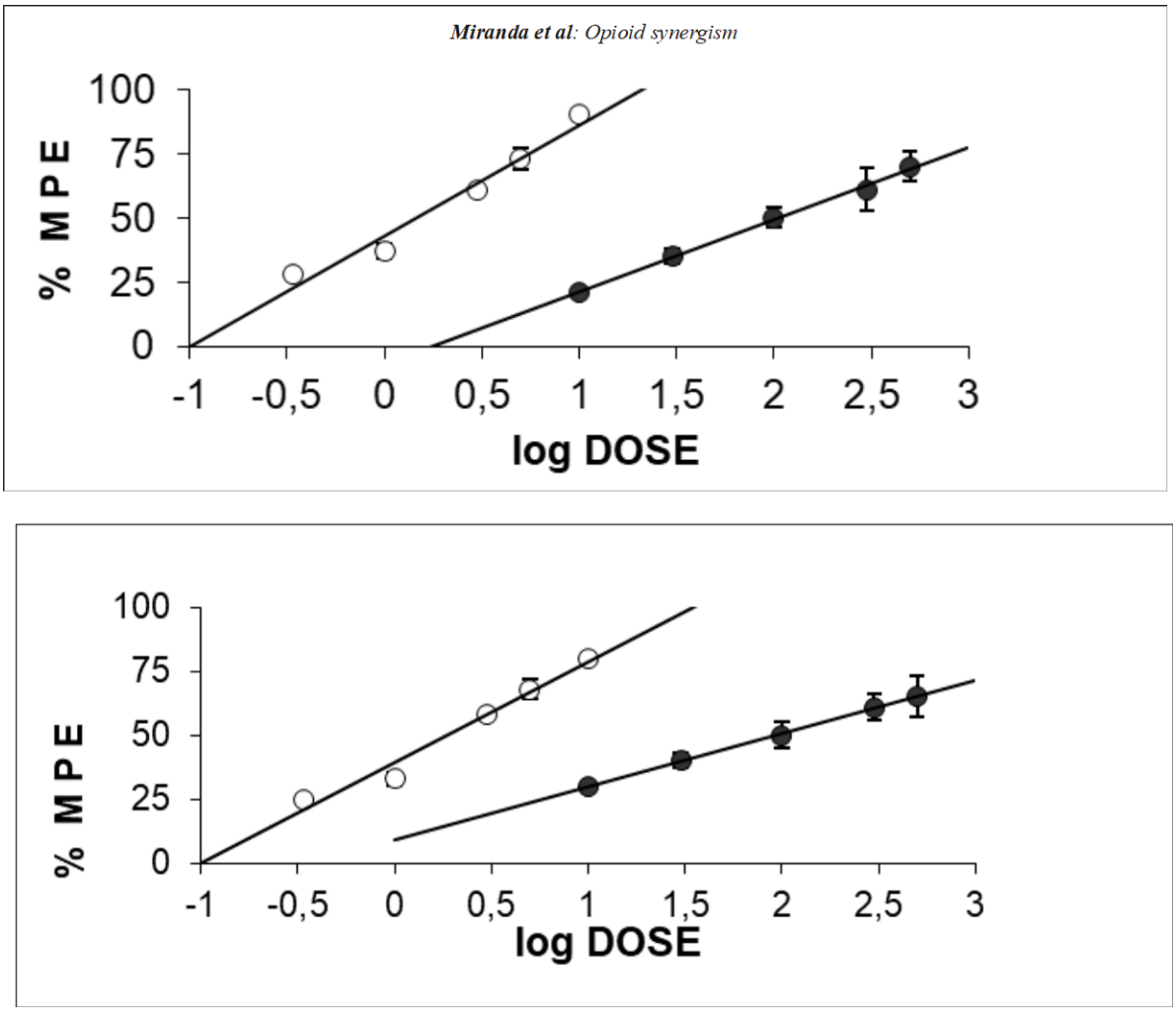


Figure 1. Dose-response for the antinociception induced by morphine () and fentanyl () in the tail flick (TF) and formalin hind paw, phase I and II (FHP-I and FHP-II) tests of mice. Each point is the mean of 6-8 mice. % MPE is the antinociception evaluated as percentage of maximum possible effect. Abscissa is log of dose of fentanyl or morphine

Analysis of interaction morphine with fentanyl

The i.p. coadministration of morphine with fentanyl, in a fixed relation 1:1 of the ED_{50}, produces a dose response in all experimental conditions. The isobolograms demonstrated that the combination, in both tests, resulted in a synergistic interaction of different magnitude, as can be seen in Table 2 and 3 and Figures 2,3 and 4.

Table 2. ED_{50} values with SEM in mg/kg for the antinociceptive activity of morphine (MOR) and fentanyl (FENTA) in algesimeter tests of mice before and after the effect of naltrexone (NTX), naltrindole (NTI) and nor-binaltorphimine (nor-BNI).

DRUGS	TF	FHP-I	FHP-II
MOR control	5.01 ± 0.91	0.17 ± 0.05	0.38 ± 0.01
MOR plus NTX	7.03 ± 0.43	0.58 ± 0.04	0.55 ± 0.03
MOR plus NTI	8.12 ± 0.91	0.61 ± 0.02	0.60 ± 0.02
MOR plus nor-BNI	8.41 ± 0.95	0.98 ± 0.06	0.67 ± 0.03
FENTA control	0.069 ± 0.006	0.055 ± 0.003	0.033 ± 0.001
FENTA plus NTX	0.099 ± 0.011	0.096 ± 0.004	0.065 ± 0.003
FENTA plus NTI	0.098 ± 0.019	0.086 ± 0.002	0.071 ± 0.008
FENTA plus nor-BNI	0.120 ± 0.009	0. 091 ± 0.006	0.097 ± 0.003

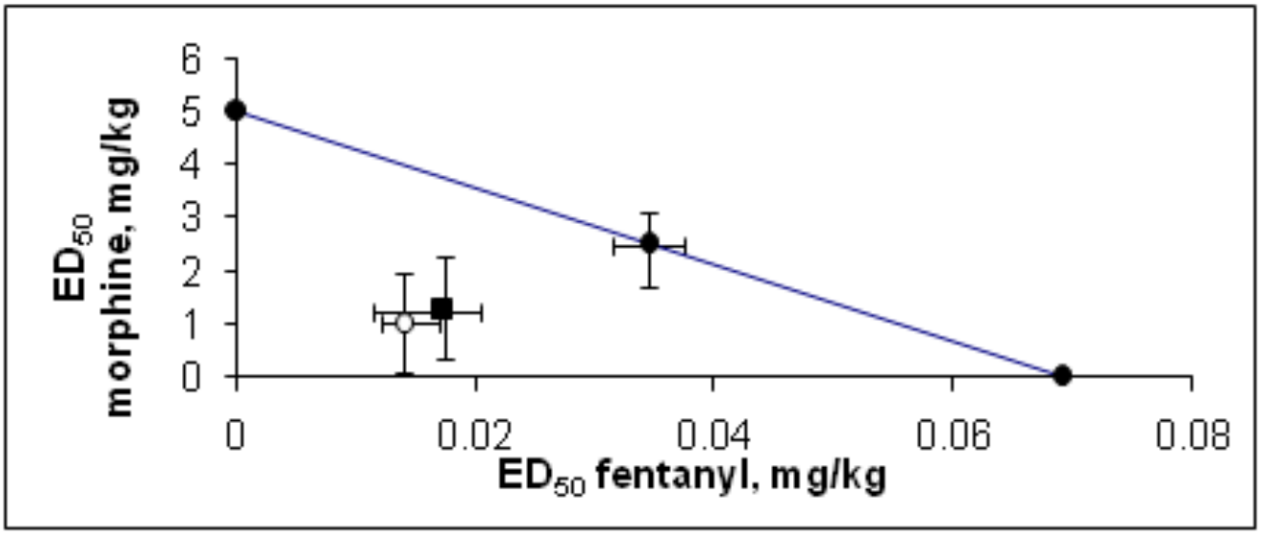
The number of mice used in each group was 12. All results obtained are significant different ($P < 0.05$) from the control group.

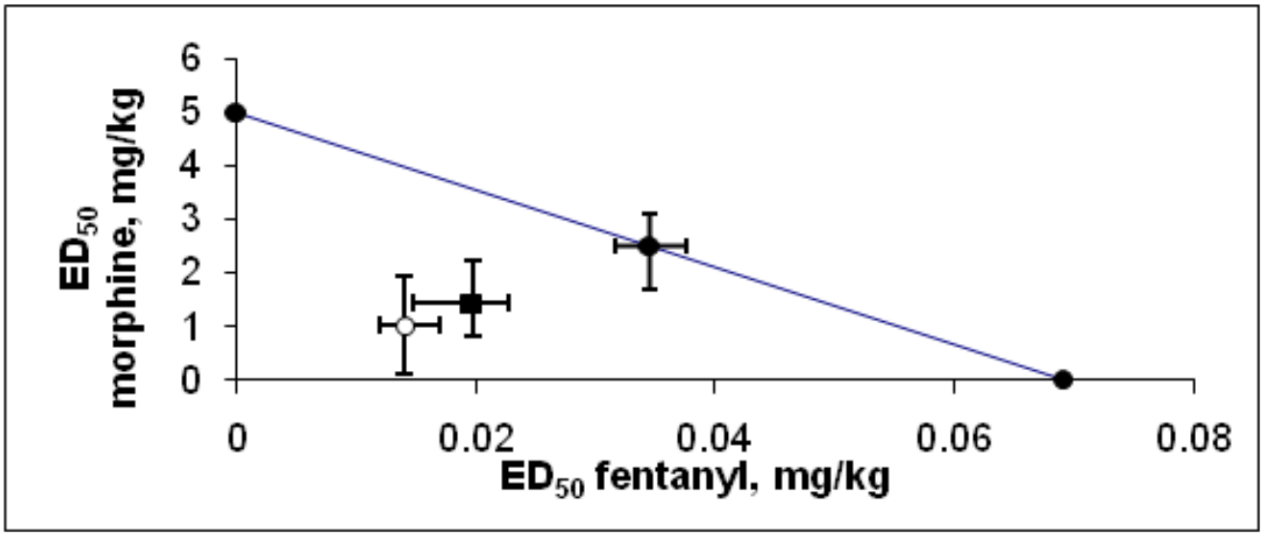
Table 3. Theoretical and experimental ED_{50} values with SEM in mg/kg and interaction index (I.I.) values for the antinociceptive interaction of morphine with fentanyl (M/F) in algesimeter tests of mice.

M/F	ED50 ± SEM THEORETICAL	XPERIMENTAL	I.I.	Interaction
TF	2.53 ± 0.05	1.03 ± 0.02	0.406	Synergistic
FHP-I	0.11 ± 0.01	0.025 ± 0.002	0.223	Synergistic
FHP-II	0.20 ± 0.01	0.035 ± 0.008	0.169	Synergistic

TF: tail flick, FHP-II: formalin hind paw, phase I, FHP-1: formalin hind paw, phase II.

Besides, the degree of potency of the mixture, were complemented by similar modification of according the interaction index, revealed the ED_{50} value of the mixture from theoretical to following rank: formalin hind paw, phase II > tail experimental (see Table 2 and 3).
flick > formalin hind paw, phase I. These changes





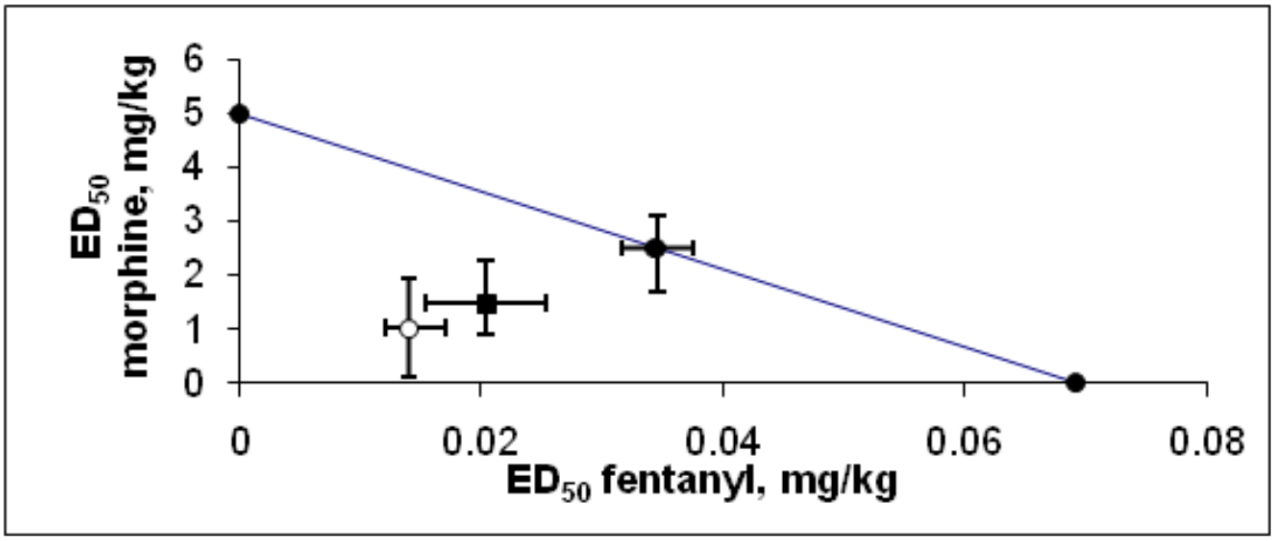
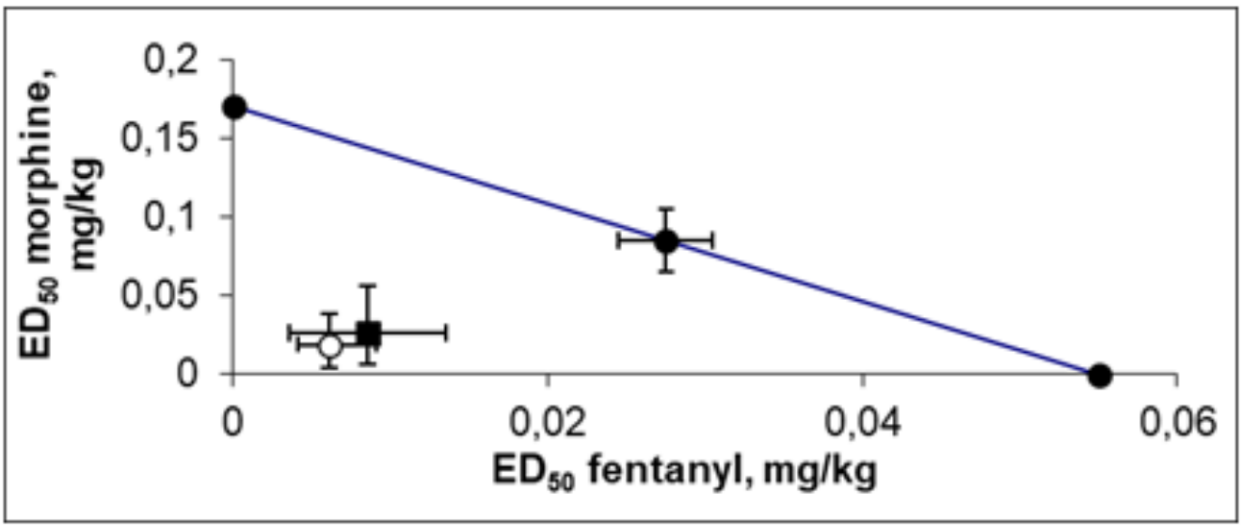
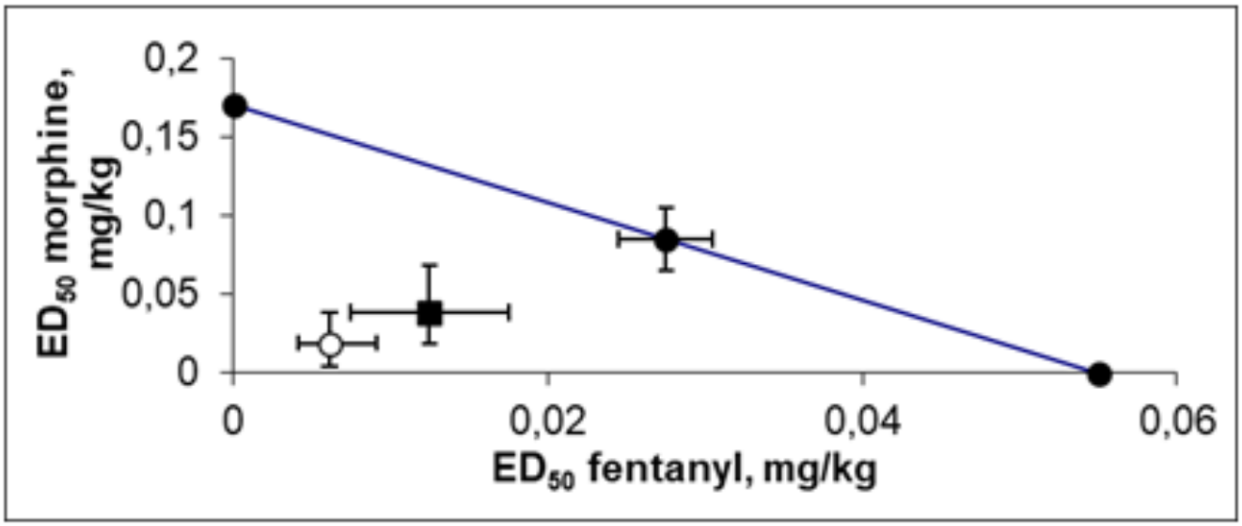


Figure 2. Isobolographic representation of the antinociceptive activity of the i.p. coadministration of fentanyl with morphine in the tail flick (TF) assay of mice after pretreatment with naltrexone (NTX), naltrindole (NTI) or nor-Binaltorphimine (nor-BNI) () indicates the theoretical ED_{50} with 95 % confidence limits (CL) and () indicates the experimental ED_{50} with 95 % confidence limits (CL).





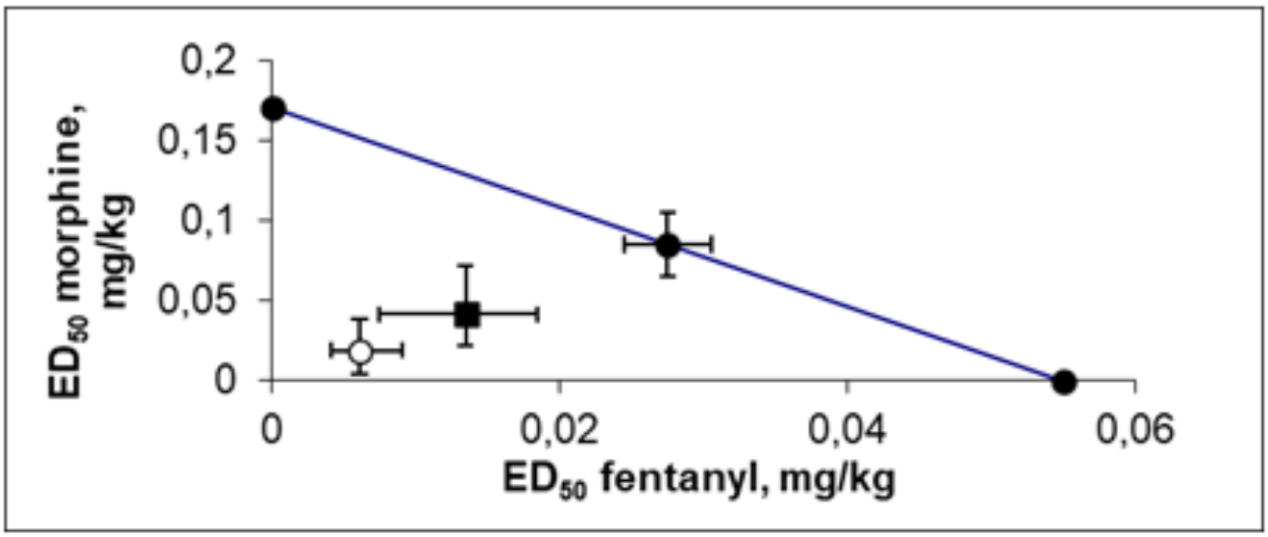
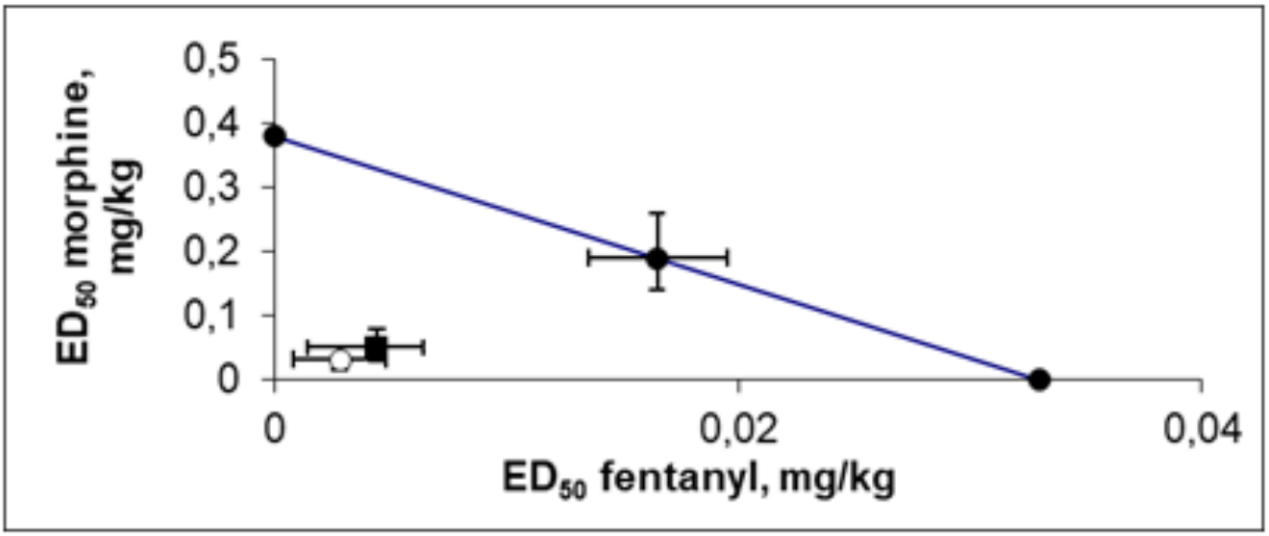
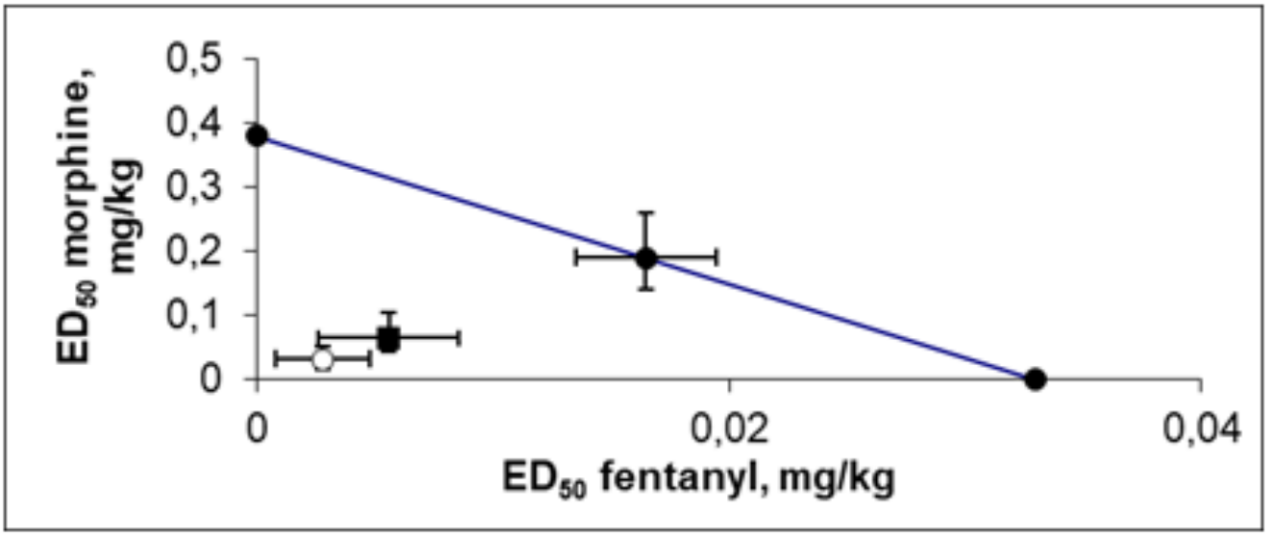


Figure 3. Isobolographic representation of the antinociceptive activity of the i.p. coadministration of fentanyl with morphine in the formalin hind paw, phase I (FHP-I) assay of mice after pretreatment with naltrexone (NTX), naltrindole (NTI) or nor-Binaltorphimine (nor-BNI) () indicates the theoretical ED_{50} with 95 % confidence limits (CL) and () indicates the experimental ED_{50} with 95% confidence limits (CL).





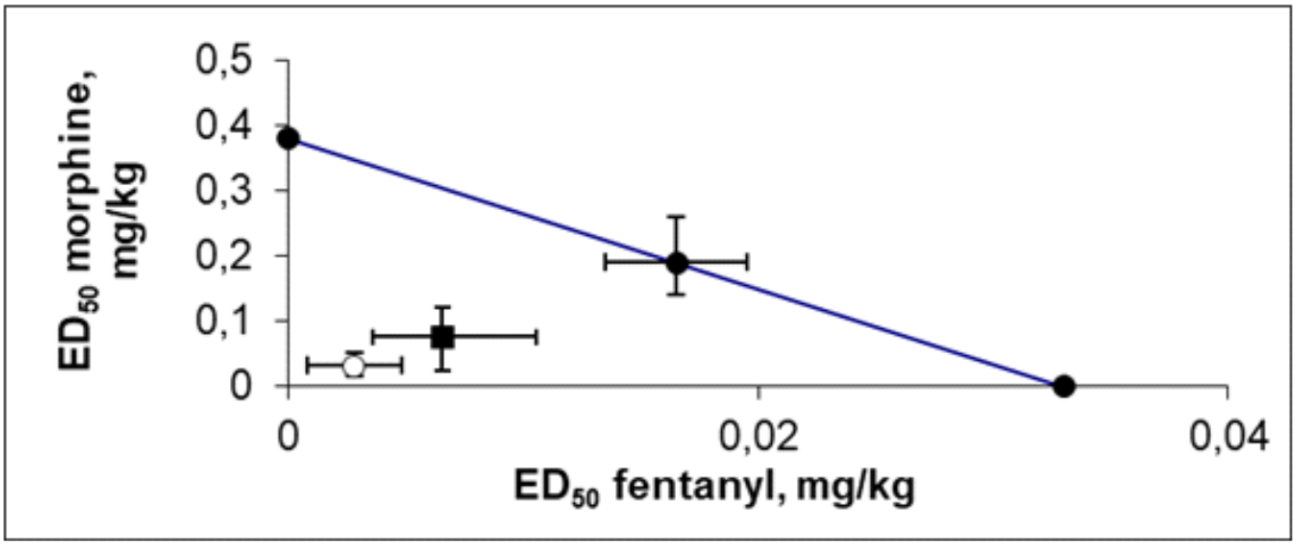


Figure 4. Isobolographic representation of the antinociceptive activity of the i.p. coadministration of fentanyl with morphine in the formalin hind paw, phase II (FHP-II) assay of mice after pretreatment with naltrexone (NTX), naltrindole (NTI) or nor-Binaltorphimine (nor-BNI) () indicates the theoretical ED_{50} with 95 % confidence limits (CL) and () indicates the experimental ED_{50} with 95% confidence limits (CL).

DISCUSSION

Opioids are widely used in the treatment of pain; however, antinociception induced by them has not been extensively studied in different animal pain models. Furthermore, most of the major studies have been conducted with isolated opioids, but have not been used in combination, as suggested in multimodal analgesia. The current work demonstrated that morphine and fentanyl induce antinociception in formalin hind paw and tail flick tests, with different potency, in which the fentanyl had a higher effect than morphine, in all tests. This result is consistent with the pharmacological properties of fentanyl: a recognized opioid with an analgesic potency of about 100 times that of morphine. Furthermore, fentanyl has a different binding capacity, expressed in nmol, from 0.7 to 1.9, compared to morphine from 1.02 to 4.1 (Zaveri et al. 2001, Waldhoer et al. 2004).

On the other hand, the results of this study are consistent with previous studies, which demonstrate that both morphine and fentanyl are drugs capable of inducing analgesia in different animal pain tests, such as acetic acid writhing, formalin hind paw, hot plate and tail flick assays (Romero et al. 2010, Miranda et al. 2007; 2012; 2013; 2019; 2020; Noriega et al. 2020).

Co-administration of morphine with fentanyl displayed synergistic pharmacological interaction in the formalin hind paw and tail flick assay. This effect, measured by the interaction index, was more powerful in the tail flick and less potent in phase II of the formalin hind paw tests. The present finding is in agreement with the basis of pharmacological synergism, which suggests that this event occurs when two drugs that produce a similar effect but have a different mechanism of action are co-administered. The described interaction could be attributed to various levels of cellular function, among which pain receptors, messengers or mediators can be mentioned. Opioids are known to interact with specific opioid receptors (MOR, DOR, KOR, NOP) with different selectivity and a large number of pharmacological and biochemical studies have demonstrated the existence of modulatory interactions between opioid and receptors. It has been suggested that morphine antinociception was preferentially mediated through MOR. Furthermore, it is believed that most clinical opioids they exert their analgesic and antinociceptive effects through MOR. Opioids may show different efficiencies probably due to MOR receptor subtypes. In relation to

receptors, 5 splice variants of the mouse-MOR have been described, the functional consequences of which could explain the greater effectiveness of fentanyl on morphine (Pasternak, 2004; Zelcer et al. 2005; Ananthan, 2006).

The current study shows that fentanyl is a more effective antinociceptive opioid compared to morphine. These disparities could be explained according the suggestion that some opioids could be acting through other targets, but this has not been expansively tested. In a study with several opioids using radioligand binding and functional activity assays, it was found novel interactions, including monoamine transporter activation. In addition, it has been reported, the interaction of morphine with α2-adrenoceptors (α2A, α2B and α2C), in contrast, fentanyl did not display affinity to α2-adrenoceptors, this effect may have an impact on the pharmacological actions of morphine. These antecedents indicate that there are interactions of opioids with other receptors that could explain the differences between the antinociception produced by morphine and fentanyl in the present work (Sirohi et al. 2008; Höcker et al. 2009; Keith et al. 2019).

CONCLUSION

In the present study, the efficacy of fentanyl in the formalin hind paw and tail flick tests was found to be greater than that of morphine. The bigger effectiveness of fentanyl could lie in the different binding capacity or in the possibility of regulation or activation of opioids and non-opioid receptors. Furthermore, co-administration of morphine with fentanyl induces synergism in murine trials, confirming the antinociceptive and anti-inflammatory capacity of both opioids

Conflict of Interests

The authors declare that they have no conflict of interests

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