SYNERGISM BETWEEN MORPHINE WITH FENTANYL IN EXPERIMENTAL MURINE ASSAYS

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 > acetic acid writhing > 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 the suggestion that opioids could be acting through other targets either by different binding capacity, by the regulation or activation of non-opioid receptors. Furthermore, co-administration of morphine with fentanyl induces synergism in all murine trials, confirming the antinociceptive and anti-inflammatory capacity of the opioids.

KEYWORDS*:* Fentanyl; Morphine; Antinociception; Anti-inflammation, Synergism

INTRODUCTION

In the therapy of various types of pain, opioids are among the most effective pain relievers available. Furthermore, these drugs have demonstrated analgesic efficacy in different animal models of pain. These tests include models of tonic pain: acetic acid and formalin writhing test and phasic pain tests: tail movement and hot plate. All of them can measure analgesia and anti-inflammation.

Opioids comprise a variety of drugs both natural (morphine, codeine) and synthetic (fentanyl, methadone, heroin), all of which are powerful and effective pain relievers, but with high potential for dependency and abuse. Opioids activate specific trans-membrane G protein-coupled receptors known as MOR, KOR, DOR, and NOP, which are found predominantly in the CNS and also in the SNP. Activation of opioid receptors induces, among other signals, inhibition of adenylate cyclase, decreased opening of calcium channels, increased potassium currents, and activation of protein kinase C. These intracellular actions lead to decrease cellular excitability and consequently neurotransmission. There are endogenous natural ligands for opioid receptors such as β-endorphins, encephalin, dynorphins, and nociceptin/orphanin FQ. Opioids have a variety of effects, including pain relief, euphoria, drowsiness, sedation, dizziness, nausea, vomiting, constipation, physical dependence, tolerance, addiction and respiratory depression (Rosenblum et al, 2008, Corder et al, 2018).

The opioids can be classified on the basis of their chemical structure as (1) opium alkaloids or opiates: codeine, morphine), (2) semisynthetic derivatives of the natural alkaloids: hydrocodone, hydromorphone, oxycodone, buprenorphine, and (3) synthetic opioids such as fentanyl, alfentanil, sufentanil, remifentanil, propoxyphene,

dextropropoxyphene, methadone, diphenoxylate, loperamide, pentazocine, butorphanol, nalbupine, levorphanol, tramadol and the opioid antagonists as nalmefene, naloxone and naltrexone, naltrindole, nor-binaltorphimine (Patham and Williams, 2012).

From the different opioids, due to their frequent use, they deserve to be highlighted: (A) Morphine is a natural opioid with a potent analgesic effect used in clinical medicine for almost two hundred years. Morphine acts by activation of MOR type receptors in the CNS and PNS. (B) Fentanyl is a synthetic opioid with similar pain activities with morphine, but is 50 to 100 times more potent. Like morphine, fentanyl is an agonist at MOR type receptors in the CNS and PNS. Fentanyl is effective in anesthesia induction as well as maintenance.

There is a method of combining analgesic drugs, each with a different mechanism of action, called multimodal analgesia. This approach should be used when treating pain reactive to the action of a single analgesic, which leads, for example in the case of opioids, to a potential reduction in undesirable effects such as nausea, vomiting, respiratory depression and also provide relief of pain using different routes of cellular action. When two drugs are taken together if the resulting response can be greater than the sum of the individual response, the interaction between the drugs 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 12 h 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 1 h before testing, used only once during the protocol, and euthanized immediately after the algesimeter test by one intraperitoneal (i.p.) injection of 60 mg/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 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 as follows:

%MPE= (latency postdrug – latency control)/(8– latency control)x 100

(B) the formalin hind paw 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 show 2 clear cut-periods, each asociated to a different type of pain. Phase I corresponding to the 5 min immediately after formalin injection and reflects tonic acute pain and phase II, spans 10 min, 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 as follow:

% MPE = 100 – [(100 x post drug licking time)/control licking time)]

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 tail flick and formalin hind paw tests of mice using at least 6 animals for each at least 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 opioids 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 posttest 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. 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: phase I of formalin hand paw > phase II of formalin hind paw > tail flick. In fentanyl the order of analgesic ratio was: formalin hind paw, phase II > formalin hind paw, phase I > tail flick. When comparing the power of the NSAIDs, the rank order of potency was: formalin hind paw phase I > formalin phase II > tail flick. All data are shown in Table 1.

Analysis of interaction morphine with fentanyl

The i.p. coadministration of morphine with fentanyl, in a fixed relation 1:1 of their ED_{50}, produces a dose response in all experimental conditions. The isobolograms demonstrated that the opioids combination, in the formalin hind paw and tail flick tests, resulted in a synergistic interaction of different magnitude, as can be seen in Table 2 and 3 and Figures 2,3 and 4. Besides, the degree of potency of the mixture, according the interaction index, revealed the following rank: formalin hind paw, phase II > tail flick > formalin hind paw, phase I. These changes were complemented by similar modification of ED_{50} value of the mixture from theoretical to experimental (see Table 2 and 3).

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 study demonstrated that morphine and fentanyl are capable of inducing antinociception in formalin hind paw and tail flick tests, with different antinociceptive potency, in which the fentanyl had a higher effect than morphine, in all tests. These results are consistent with the pharmacological characteristics of fentanyl: a recognized greater analgesic potency than around morphine 100 times. Furthermore, fentanyl has a different binding capacity, expressed in nmol, from 0.7 to 1.9, compared to morphine from 1.02 to 4 (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. 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. These results are in agreement with the general basis of synergistic pharmacological interactions, which suggest that this effect occurs if two drugs are co-administered that induce the same effect but have a different mechanism of action. In the present work, the described interaction could be attributed to various levels of cellular function, among which pain receptors, messengers or mediators can be mentioned or others. 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 comprehensively tested. A study with several opioids using radioligand binding and functional activity assays, it was found novel interactions, including monoamine transporter activation. In addition, t 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 the

tail flick assays, was found to be relatively greater compared to the morphine result. 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 all murine trials, confirming the antinociceptive and anti-inflammatory capacity of both opioids.

Acknowledgement

None

Conflict of Interests

The authors declare that they have no conflict of interests

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LEGENDS TO FIGURES

FIGURE 1. Dose-response for the antinociceptive avtivity induced by morphine (●) and fentanyl (○) in the tail flick (TF) and formalin hindpaw, phase I and II (FHP-I and FHP-II) tests of mice. Each point is the mean of 6-8 mice. % MPE represent antinociception evaluated as percentage of maximum possible effect. Abscisa is log of dose of fentanyl or morphine.

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).