**SYNERGISTIC INTERACTION BETWEEN DEXKETOPROFEN AND FENTANYL IN MURINE FORMALIN ASSAY**

**Abstract**

Nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids are between the best therapeutic possibilities in the treatment of pain. If they use in combination may increase their analgesic profile with a reduction in the dose and adverse effects. The aim of this study was to evaluate the pharmacological interaction of the NSAIDs, dexketoprofen (DEX), with the opioid, fentanyl in the experimental test of formalin hind paw (FHP) of mice.Antinociception was assessed by dose–response curves to dexketoprofen and fentanyl and results are presented as means ± SEM and differences were calculated by one-way ANOVA followed by Tukey's post-test. The i.p. administration of DEX or fentanyl, isolated, produced dose related antinociceptive effects with different potencies in FHP test. The nocifensive response induced by DEX with fentanyl combined in a 1:1 ratio of their analgesic potency (ED50) was synergistic. The synergism induced by the coadministration of DEX with fentanyl in the mice FHP assay suggests that only COX inhibition and MOR opioid receptor activation are a partial explanation for the analgesic potency of DEX and fentanyl. Activation of different antinociceptive pathways is likely to contribute to the action of these drugs. These results suggest that the combination of DEX and fentanyl in a 1:1 ratio could be a new future alternative for pain pharmacotherapy.

**Keywords**: Dexketoprofen, Fentanyl, Synergism, Isobolograms, Formalin test

**Introduction**

Dexketoprofen (DEX), a nonsteroidal anti-inflammatory drug (NSAIDs) and fentanyl, an opioid agent are effective drugs for the pharmacotherapy of several types of pain. DEX induce antinociception when used, either in animal models or in humans (Romero et al. 2010; Noriega et al., 2022; Kuczyńska et al. 2022; Shafi 2022).

DEX is the (S+) enantiomer of racemic ketoprofen, with analgesic, anti-inflammatory, and antipyretic properties (Shafi 2022). The mechanism of action of the drug, both for efficacy and for adverse effects, is the inhibition of cyclooxygenase isoenzymes (COXs): COX-1 and COX-2, which limit the synthesis of prostanoids. DEX is a non-selective COXs inhibitor but effective at low doses, since as single isomer of ketoprofen permits to reduce by 50 % its effective dose and not produce serious adverse effects. Also, it has been reported that DEX is a better analgesic either administered alone as in combination (Kuczyńska et al. 2022).

Opioids are the most effective and widely used drugs for the treatment of severe pain; however, unwanted side effects limit their use. It may be note that opioids are paradoxically both analgesic and hyperalgesic. Opioids active specific receptors: mu-opioid (MOR), delta-opioid (DOR), kappa-opioid (KOR) and nociceptin-peptide-opioid (NOP). Activation of opioid receptors induces, among other actions, inhibition of adenylate cyclase, activation of protein kinase C, changes in calcium channels and potassium conductance. All these intracellular actions produce a variety of effects, include pain relief, euphoria, constipation, tolerance, physical dependence, addition (Corder et al, 2022; Miranda et al., 2022). Of the various opioids, fentanyl deserves mention as an MOR receptors agonist and of synthetic in nature, it is 50 to 100 times more potent than morphine and 25 times than heroin and also used in anaesthesia. It is an opioid of rapid absorption and effect (Shafi 2022).

Due to the complex scheme underlying pain, pharmacotherapy of available treatments with an analgesic alone sometimes does not provide effective pain control and limited by their efficacy and side effects. However, there is multimodal analgesia, which consists of the use of two or more drugs to improve the efficacy of pain treatment. It is intended with this technique to have a synergistic or at least additive analgesic effect accompanied by a reduction in dose and side effects of each of the components.

There are combinations of opioids with NSAIDs that produce synergistic interactions and are used clinically: paracetamol with tramadol; morphine with paracetamol; dexketoprofen with morphine and others. Also, there are some reports studying synergy using isobolographic analysis in algesimetric animal models (Freo, 2022).

The aim of the present work was to evaluate the type of interaction induced by the co-administration of a non-selective NSAID (dexketoprofen) with a selective MOR opioid agonist (fentanyl) by isobolographic analysis using an algesimetric chemical assay of hind paw formalin (FHP).

**Materials and methods**

**Animals**

Male CF-1 mice (25–30 g) from the Central Animal Facility of the Universidad de Chile Faculty of Medicine were used. Animals were kept under a 12-h light–dark cycle at 22 ± 1° C with free access to food and water (ad libitum). All animal procedures were made in accordance with the rules of the International Association for the Study of Pain and approved by the Animal Care and Use Committee of the Faculty of Medicine (CBA 0852/FMUCH/2018). Mice were acclimatized to the laboratory for at least 1 h before testing, used only once during the protocol, and euthanized after the algesimeter test with an intraperitoneal (i.p.) injection of 60 mg/kg of pentobarbital. The minimum number of animals required to establish consistent effects of the drug treatment was used.

**Measurement of antinociceptive activity**

Antinociception was assessed by the formalin hind paw (FHP) test as described previously (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 pain was assessed as the time spent licking or biting the injected paw, expressed in seconds, and converted to % MPE. The test shows two phases, each associated to a different type of pain. Phase I spans the first 5 min following the formalin injection and reflects tonic acute pain. Phase II spans 10 min, starting 20 min after formalin injection and reflects inflammatory pain. The control values for Phases I and II were 116.3 ± 7.4 s (n = 12) and 145.6 ± 9.3 s (n = 12), respectively.

**Experimental design**

The antinociceptive activity of dexketoprofen and fentanyl was evaluated from dose response curves obtained by i.p. administration 30 min prior to test. The method of isobolographic analysis was used to evaluate the interaction between DEX and fentanyl with the method previously described (Miranda 2001).The isobologram is a graphical representation of isoeffective doses of DEX or fentanyl combined in fixed ratios (1:1) of the corresponding ED50, which was determined in isolation for each drug. In summary, the isobolograms, were constructed connecting ED50 of DEX in the abscissa with the ED50 of fentanyl in the ordinate to obtain the additive line. For each drug combination, the experimental ED50 was obtained which was compared with the ED50 attained theoretically and denoted by a point on the additive line. The point representing the experimental ED50 will be located in the isobolograms, and the site of the graph where the experimental point is located determines the type of interaction. If the experimental point is below the line of additivity and is statistically different from the point of additivity, the effect of the combination of opioids is synergistic or supraadditive. To certify the nature of the mixture of the drugs, the interaction index (I.I.) was also calculated with the formula I.I. = experimental ED50/theoretical ED50. If the I.I. value is lesser to 1, the interaction is synergistic.

**Drugs**

Drugs were freshly dissolved in sterile physiological saline solution of 10 ml/kg, for i.p. administration. Dexketoprofen and fentanyl were y provided by local laboratories.

**Statistical analyses**

Results are presented as means ± standard error of the mean (SEM). The statistical differences between the results were assessed by one-way analyses of variance (ANOVA) followed by Tukey’s post-test; P values less than 0.05 (P < 0.05) were considered to reflect statistically significant differences. Statistical analyses were carried out using the program Pharm Tools Pro, version 1.27, McCary Group Inc., PA, USA.

**Results**

 **Antinociception induced by DEX and fentanyl**

The i.p. administration of DEX or fentanyl produced dose related antinociceptive effects with different potencies in FHP test. Besides, the administration i.p. of fentanyl produced parallel dose-response curves in both phases of the FHP test, however, a similar result was not obtained with the DEX dose-response curves.

Fentanyl administration induced the highest relative potency, expressed as ED50, in both phases of FHP test compared to DEX. In Phase I it was 277 times and in Phase II it was 1.710 times. These results are shown in table 1 and depicted graphically in Figure 1.

**Isobolographic analysis of interaction DEX with fentanyl**

In the FHP test the nocifensive response induced by DEX with fentanyl combined in a 1:1 ratio of their analgesic potency (ED50) was synergistic, as can be seen in Fig. 2.

 The data generated by the isobolograms corresponding to the ED50´s theoretical and experimental together with the interaction index (I.I.) are shown in table 2.

**Discussion**

The results obtained in the current study show the antinociceptive activity of DEX and fentanyl, in the formalin test in mice, is dose-dependent and with different potencies in each of the test phases. On the other hand, this finding confirms that opioids and NSAIDs show a different profile in nocifensive activity.

Thus, opioid (fentanyl) has greater efficacy than NSAID (DEX). Another difference is reflected in the dose-response curves of each phase, being those of fentanyl parallel, but not those of DEX. This difference could be attributed to the fact that the parallelism of the dose-response curves is consistent with a common mechanism of action (Miranda 2006).

The outcomes in relation with antinociception obtained by the drugs in this study, when are administered isolated, confirm previous findings that fentanyl or DEX elicits antinociception in this trial (Romero et al, 2010; Isordia et al., 2011; Miranda et al., 2012; Nporiega et al., 2020). However, it is necessary to highlight that the cited results report an effect of NSAIDs in both phases of the FHP, an event that does not agree with the reports that indicate that NSAIDs only induce an effect in phase II but not in phase I of the FHP (Zhao et al., ,2017).

The synergy induced by the co-administration of DEX with fentanyl, in a 1:1 potency ratio, is consistent with the general premise of multimodal analgesia, in which it is accepted that the concurrent administration of two or more drugs with different mechanisms of action may alter the pharmacology profile of drugs in association (Miranda et al, 2001; Tallarida 2001).

The interaction synergic obtained in the present work must be based in the different pathways of action induced by each drug of combination. Thus, the antinociception produced by DEX an agent NSAIDs is primarily due to COXs inhibition, nevertheless, additional or alternative mechanisms of action should be considered, between them depletion of substance P; interaction with NO-cGMP system ATP-sensitive K+ channels; the NO-cGMP-K+ channel pathway; opioid receptors; adrenergic, cholinergic, serotonergic and glutamatergic pathways; affinity for phospholipase A2, modulation of IL-β, IL-6 and others ( Gunaydin et al 2018; Mayoral et al., 2022).

Regarding the antinociception induced by fentanyl, an opioid drug, it is mainly due to the activation of MOR receptors; however, other mechanisms of action have been reported. It has been proposed that fentanyl induces a decrease in cAMP with inhibition of the release of GABA, dopamine, acetylcholine, and norepinephrine. In addition, it is capable of inhibiting serotonin reuptake with a corresponding increase in inter-synaptic serotonin release through GABA inhibition, which leads to blocking the effect of glutamate ( Al-Hasani et al. 2011; Baldo et. al 2020).

The data presented indicates that when DEX and fentanyl are administered alone, they produce a dose-dependent antinociceptive effect of different potencies in both phases of the mouse FHP assay. Co-administration of DEX and fentanyl was found to be synergistic. These actions suggest that COX inhibition and MOR opioid receptor activation are only a partial explanation for the analgesic potency of DEX and fentanyl. Therefore, the effects of both analgesics should be associated with multiple additional mechanisms of action. Based on these findings, it is proposed that the co-administration of DEX with fentanyl could be a new and effective alternative for pain management.

**5. Conclusions**

Intraperitoneal administration of DEX and fentanyl, separately, produced dose-dependent antinociception with different potencies in both phases of the mouse FHP assay. When administered together, a synergistic effect is demonstrated. It seems that only COX inhibition and MOR opioid receptor activation are a partial explanation for the analgesic potency of DEX and fentanyl. Activation of different antinociceptive pathways is likely to contribute to the action of these drugs. These results suggest that the combination of DEX and fentanyl in a 1:1 ratio could be a new future alternative for pain pharmacotherapy.

**Abbreviations**

**NSAIDs:** Nonsteroidal anti-inflammatory drugs

**FHP:** formalin hind paw

**DEX:** dexketoprofen

**ED50:** dose that induce 50% effect maximun

**S+:** isomer with left-handed configuration

**COX:** ciclooxigenase

**MOR:** opioid receptor mu

**DOR:** opioid receptor delta

**KOR:** opioid receptor kappa

**NOR:** opioid receptor nociceptine peptide

**I.I.:** Interaction index

**NO-cGMP:** nitric oxide-cyclic guanosine monophosphate

**ATP:** adenosine triphosphate

**IL-β:** interleukine 1β

**IL-6:** interleukine 6

**cAMP:** cyclic adenosine monophosphate

**GABA:** gamma aminobutyric acid

**Declarations**

**Ethics approval and consent to participate**

 All animal procedures were made in accordance with the rules of the International Association for the Study of Pain and approved by the Animal Care and Use Committee of the Faculty of Medicine (CBA 0852/FMUCH/2018).

**Consent for publication**

Not applicable

**Availability of data and materials**

Data of this work are available after rational request from the corresponding author

**Competing interests**

The authors declare that the research was conducted without potential conflict of interest.

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**Authors’ contributions**

All authors contributed directly and substantially to the study and approved the final version of the manuscript

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