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Synergism between dexketoprofen and meloxicam in an orofacial formalin test was not modified by opioid antagonists

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Abstract:
Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used drugs for the management of acute and chronic pain. The role of the opioid system in the synergism between NSAIDs is not well characterized. Mice were injected with a 5% formalin solution (20 μl) into the upper right lip to perform an orofacial formalin test. The isobolographic method was used to determine the interaction between dexketoprofen, which is the (S)-(+) enantiomer of ketoprofen, and meloxicam co-administration. Additionally, the non-selective, opioid antagonist naltrexone, the selective δ opioid receptor (DOP) antagonist naltrindole and the selective κ opioid receptor (KOP) antagonist norbinaltorphimine were used to assess the opioid effects on this interaction. Intraperitoneal administration of dexketoprofen or meloxicam induced dose-dependent antinociception with different phase I and phase II potencies in the orofacial formalin test. Meloxicam displayed similar potencies (ED_{50}) in phase I (7.20 mg/kg) and phase II (8.60 mg/kg). Dexketoprofen was more potent in phase I (19.96 mg/kg) than in phase II (50.90 mg/kg). The interactions between dexketoprofen and meloxicam were synergistic in both phases. This was determined based on the fixed ratios (1:1) of their ED_{50} values, which were determined by isobolographic analysis. Furthermore, this antinociceptive activity does not seem to be modulated by opioid receptor blockers because they did not induce changes in the nature of this interaction. This finding may be relevant with regards to NSAID multi-modal analgesia where an opioid antagonist must be used.

Key words:
algesiometric tests, antinociception, isobolographic analysis, synergism

Introduction

Exaggerated or diminished effects will sometimes occur when drugs with similar effects are used concurrently [24]. In certain cases, co-administering antinociceptive agents results in synergistic effects; therefore, the doses of each drug can be reduced [18].

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used classes of drugs for the management of acute and chronic pain. They pre-

vent the development of inflammation and produce their analgesic effects by blocking the synthesis of prostaglandins (PGs) in the periphery by inhibiting cyclooxygenase enzymes. These enzymes catalyze PG synthesis from arachidonic acid. Three isoforms of cyclooxygenase (COX) have been identified: COX-1 (expressed constitutively throughout the body), COX-2 (expressed predominantly in inflammatory processes) and COX-3 (a recently identified isoform that is mainly located in the human cerebral cortex). NSAIDs provide excellent analgesia for mild to moderate pain. They are particularly useful in the initial management of pain with an inflammatory component [21].

Opioids are the most effective drugs used in treating severe pain, and they exert their actions by interfering with pain in the central nervous system [11]. However, unwanted side effects may seriously limit their clinical use. Combinations of opioids and COX-2 inhibitors have shown synergistic interactions and are in clinical use for postoperative pain [12, 14]. To date, four opioid receptors have been cloned: MOP (μ for morphine), KOP (for ketocyclazocine), DOP (δ for deferens; it was first identified in mouse vas deferens) and NOP (for nociceptin) [28]. However, there is a disparity between the existences of only four opioid receptor genes and the substantial pharmacological evidence for additional opioid receptor phenotypes.

Few reports have studied the synergy between COX-1 and COX-2 inhibitors using isobolographic analysis in acute and inflammatory orofacial pain. The purpose of the present study was to assess the interaction between the (S)-(+) enantiomer of racemic ketoprofen (dexketoprofen), which is a COX-1 inhibitor [15] that inhibits PG activity, and a selective COX-2 inhibitor (meloxicam) in a modified formalin orofacial model [19]. In addition, we assessed the effects of opioid receptors on this interaction.

Materials and Methods

Male CF-1 mice (35–40 days old, weighing 29 ± 1.5 g) were housed in a 12 h light-dark cycle at 22 ± 1°C, and they had free access to food and water. The animals were acclimatized to the laboratory environment for at least 2 h before the experiments began. Experiments were carried out in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals, and the Institutional Animal Care and Use Committee at the University of Chile (Santiago, Chile) approved all experimental procedures. Each animal was used only once and received only one dose of the drugs tested. All drugs were freshly prepared in normal saline and administered intraperitoneally (*ip*). All observations were performed by the authors in randomized and blinded manners. Control animals were given saline and were run interspersed concurrently with the drug-treated animals (at least two mice per group) to prevent the controls from being run in a single group at one time.

Orofacial formalin test

The method described by Luccarini et al. [13] was used for the orofacial formalin test with modifications. To perform the test, a 5% formalin solution (20 μl) was injected into the upper right lip of each mouse with a 27-gauge needle. In the preliminary experiments, different groups of mice were treated with different formalin concentrations (1, 2 or 5%) to establish the concentration-response relationships for both phases. Based on these results, we selected the 5% formalin dose because inhibition was easy to detect. After the formalin injection, mice were immediately returned to a glass observation chamber. The degree of pain intensity was assessed by the total time that the animal spent rubbing its lip with one of its extremities. Administration of the analgesics (or saline solution for the control group) and the opioid receptor blockers occurred 30 min and 1 h, respectively, before formalin administration. Two phases were distinguished during the assay. Phase I corresponded to the 5-min period starting immediately after formalin injection that represents tonic acute pain due to peripheral nociceptor sensitization. Phase II was recorded as the 10-min period starting 20 min after formalin injection and represents inflammatory pain. Drug effects were characterized after the administration of at least four doses in logarithmic increments. The maximum possible effect (MPE) was calculated as follows:

$$\% \text{ MPE} = 100 - [\text{post drug rubbing time/control rubbing time} \times 100]$$

The dose that produced 50% of the MPE (ED_{50}) was calculated from the linear regression analysis of the curve that was obtained by plotting the log dose *vs.* % MPE.