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Analgesic synergism between intrathecal morphine and cyclooxygenase-2 inhibitors in mice

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Abstract

The analgesic effects of the intrathecal coadministration of morphine with nimesulide, meloxicam and parecoxib, preferential cyclooxygenase-2 (COX-2) inhibitors, were studied in mice using a chemical model of visceral pain, the acetic acid writhing test. Isobolographic analysis was used to characterize the interactions between mixtures of morphine with each non-steroidal anti-inflammatory drug. Antinociception dose–response curves were analyzed to obtain the ED_{50}'s of each drug. A dose response curve for fixed ratio mixtures of morphine with COX-2 inhibitors was then performed and the observed ED_{50}'s were plotted on a two-dimensional isobologram. All the combinations tested showed synergistic interactions and the strength of the interaction was ranked as: morphine/parecoxib > morphine/meloxicam > morphine nimesulide. The results demonstrate that the intrathecal coadministration of COX-2 inhibitors significantly enhance morphine-induced antinociception and could result in an opioid sparing action which may be useful in the clinical treatment of severe pain. A sparing action means that less opioids have to be administered to obtain a given analgesic effect. Since intrathecal morphine is often used in clinical pain situations, the opioid sparing effect resulting from the synergy observed with the coadministration of COX-2 inhibitors may be clinically relevant. One of the most significant advantages should be the reduction of opioid toxicity which often acts as a major obstacle in pain treatment.

Keywords: Analgesia; COX-2; Morphine; Synergism; Writhing test; Isobologram

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are mainstays in acute and chronic pain management and their beneficial actions have been linked to their ability to inhibit cyclooxygenases: constitutive COX-1 and inducible COX-2 (Gajraj, 2003; Warner and Mitchell, 2004). However, in the spinal cord, COX-2 immunoreactivity is present in neurons of all lamina, particularly in the superficial layers and COX-2 can be considered a constitutive enzyme (Warner and Mitchell, 2004). In addition, there is increasing evidence that NSAIDs exert their analgesic effects through a variety of other mechanisms. In the dorsal horn of the spinal cord several peptides (substance P, endorphins); aminoacids (glutamate, GABA), neurotransmitters (serotonin, norepinephrine, acetylcholine), nitric oxide and arachidonic acid metabolites are implicated in the transmission and regulation of pain information (Kroin et al., 2002; Miranda et al., 2002; Miranda and Pinardi, 2004; Pinardi et al., 2002; Sandrini et al., 2002).

Opioids are the most effective and widely used drugs for the treatment of severe pain. However, unwanted side effects may seriously limit its clinical use. Opioids can be used also intrathecally for postoperative pain control in major surgery (Fournier et al., 2000). Some combinations of opioids with COX-2 inhibitors have shown synergistic interactions and are in clinical use for postoperative pain (Raffa, 2001; Kroin et al., 2002; Malan et al., 2003). Our group has published a study in which different combinations of morphine and several NSAIDs, including acetaminophen,

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were found to be synergistic after systemic administration (Miranda et al., 2004) and Hernandez-Delgadillo et al. (2002) reported that metamizol potentiates morphine effect in visceral pain. Nevertheless, there are few reports studying synergy using isobolographic analysis in animal algesiometric models (Deciga-Campos et al., 2003).

The present work was undertaken to characterize the type of interactions between the intrathecal coadministration of morphine and the preferential or selective inhibitors of COX-2 nimesulide, meloxicam and parecoxib (Engelhardt, 1996; Famaey, 1997; Simmons et al., 2004; Padi et al., 2004; Warner and Mitchell, 2004). The interactions were evaluated by two-dimensional isobolographic analysis, using a visceral pain mice model.

2. Materials and methods

2.1. Animals

Male CF-1 mice (28–30 g), housed on a 12 h light–dark cycle at 22±2 °C and with access to food and water ad libitum were used. Experiments were performed in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigation of experimental pain approved by the Animal Care and Use Committee of the Faculty of Medicine, University of Chile. Animals were acclimated to the laboratory for at least 2 h before testing, were used only once during the protocol and were killed by cervical dislocation immediately after the algesiometric test.

2.2. Intrathecal injections

As previously described (Miranda et al., 1993), for intrathecal (i.t.) injections the animals were restrained manually and a 50 μL Hamilton syringe with a 26-gauge needle was inserted into the subdural space between L5 and L6. The doses were administered in a constant volume of 5 μL, dissolved in a slightly hyperbaric solution of glucose (6%) to limit rapid diffusion of the drugs to higher levels of the spinal cord. A flick of the tail during insertion of the needle is indicative of a successful spinal administration (Hylden and Wilcox, 1980). Control animals (6% glucose) were run interspersed concurrently with the drug treatments.

2.3. Measurement of analgesic activity

Analgesic activity was assessed by the writhing test, a chemical visceral pain model. Observations were performed in a blinded manner. Mice were injected intraperitoneally with 10 mL/kg of 0.6% acetic acid solution 15 min after the intrathecal (i.t.) administration of the drugs, a time at which preliminary experiments showed occurrence of the maximun effect. A writhe is characterized by a wave of contraction of the abdominal musculature followed by the extension of the hind limbs. The number of writhes occurring in a 5 min period was counted, starting 5 min after the acetic acid administration. Antinociceptive activity was expressed as percent inhibition of the usual number of writhes observed in saline control animals (19.8±0.30, $n=70$).

2.4. Experimental protocol

Dose–response curves for morphine (MOR), nimesulide (NIME), meloxicam (MELO) and parecoxib (PARE), were obtained using at least six animals at each of at least four doses. A least-squares linear regression analysis of the log dose–response curve of each drug allowed the calculation of the dose that induced 50% antinociception (ED_{50}). Then, a dose–response curve was also obtained by the coadministration of MOR with each NSAID ($ED_{50\ MIX}$) in fixed ratio combinations based on fractions of their respective ED_{50} values: 1/2, 1/4, 1/8, 1/16 (ratio values given in Table 2). The drugs of the combinations were dissolved and injected together in the same solution. Isobolographic analysis was used to determine drug interactions. The method has been described previously in detail (Miranda et al., 2002). Supra-additivity or synergistic effect is defined as the effect of a drug combination that is higher and statistically different ($ED_{50\ MIX}$ significantly lower) from the theoretical calculated additive equieffect ($ED_{50\ ADD}$) of a drug combination with the same proportions. If the ED_{50}'s are not statistically different, the effect of the combination is additive and additivity means that each constituent contributes with its own potency to the total effect. The interaction index is an indication of the strength of the interaction and was calculated as follows: experimental $ED_{50\ MIX}$/theoretical $ED_{50\ ADD}$. If the value is close to 1, the interaction is additive, corresponding with the additivity line of the isobologram. Values lower than 1 are an indication of the magnitude of supra-additive or synergistic interactions and values higher than 1 correspond to sub-additive or antagonistic interactions (Tallarida, 2001).

2.5. Drugs

The following NSAIDs were freshly dissolved in a slightly hyperbaric solution of glucose (6%) to limit diffusion and were provided by local pharmaceutical companies: nimesulide by Grunenthal Chilena Limited, meloxicam by Laboratorios Saval S.A. and parecoxib by Pfizer Chile. Morphine hydrochloride was purchased from Sigma Chemical Co, St. Louis, MO, USA. Doses were expressed on the basis of the salts.

2.6. Statistical analysis

Results are presented as ED_{50} values with 95% confidence limits (CL). The statistical difference between theoretical and experimental values was assessed by