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

Table 1
ED_{50} values and 95% confidence limits (CL) for the antinociceptive effect of morphine and NSAIDs administered i.t. in the writhing test of mice

Drug	ED_{50} mg/kg i.t. (CL)
Morphine	0.00018 (0.00009–0.00034)
Meloxicam	0.22 (0.19–0.25)
Nimesulide	0.29 (0.21–0.39)
Parecoxib	0.62 (0.42–0.93)

Student's *t*-test for independent means and P values <0.05 were considered significant.

3. Results

3.1. Antinociception induced by NSAIDs and morphine

The i.t. administration of NIME, MELO, PARE and MOR induced a dose-dependent antinociceptive activity with different potencies in the writhing test of mice. The

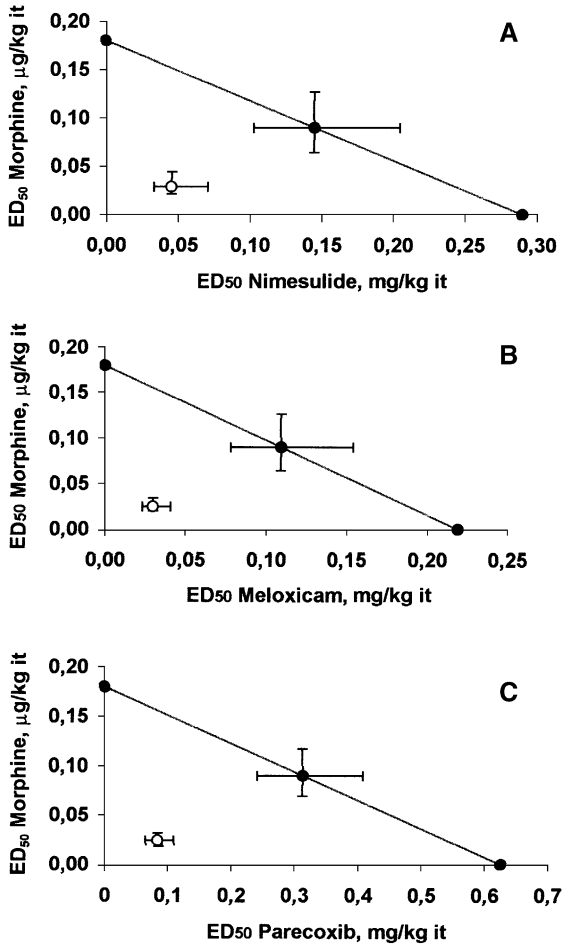

Fig. 1. Isobolograms for the intrathecal administration of the combinations morphine/nimesulide (A), morphine/meloxicam (B) and morphine/parecoxib (C). Filled circles correspond to the theoretical additive ED_{50} with 95% confidence limits and open circles correspond to the experimental ED_{50} of the mixture with 95% confidence limits. Ordinates are in µg/kg and abscissae in mg/kg.

Table 2
Theoretical and experimental ED_{50} values with 95% confidence limits (CL) and fixed ratios for combinations of NSAIDs/morphine administered i.t. in the writhing test of mice

Combination	ED_{50} (95% CL) (mg/kg)		Mixture ratio
	NSAID/morphine		
	Theoretical	Experimental	
Meloxicam/morphine	0.11 (0.10–0.12)	0.06* (0.04–0.07)	1216
Nimesulide/morphine	0.15 (0.11–0.18)	0.07* (0.05–0.11)	1611
Parecoxib/morphine	0.31 (0.24–0.40)	0.10* (0.08–0.14)	3476

* $P<0.05$.

ED_{50} values and 95% confidence limits for the antinociceptive effects of morphine and NSAIDs are shown in Table 1. As can be seen, i.t. MOR is more than 1200 times as potent as MELO, 1600 times as potent as NIME and 3400 times as potent as PARE.

3.2. Interactions between NSAIDs and morphine

The antinociceptive activity of fixed ratio mixtures of ED_{50} fractions of each NSAID with ED_{50} fractions of MOR was assessed by the analysis of the dose–response curves obtained after i.t. coadministration of the mixtures. The isobolographic analysis of the combinations MOR/MELO, MOR/NIME and MOR/PARE resulted in synergistic interactions, as can be seen in Fig. 1. Table 2 shows the experimental and the theoretical additive ED_{50} values for the combinations with their 95% confidence limits and the mixture fixed ratios. Furthermore, the interaction index values of the combinations demonstrated the following rank of strength for the combinations: MOR/NIME<MOR/MELO<MOR/PARE (Table 3).

4. Discussion

The results of the present work demonstrate that the preferential COX-2 inhibitors nimesulide, meloxicam and parecoxib possess antinociceptive activity in the writhing test of the mice, corresponding with the selectivity reported by Warner and Mitchell (2004). The combination MOR/PARE seems to be a little better than the other tested, and the comparison of the interaction index values (Table 3)

Table 3
Interaction index (I.I.) of the combinations of NSAIDs and morphine administered i.t. in the writhing test of mice

Combination	Interaction index (I.I.)
Parecoxib/morphine	0.348
Meloxicam/morphine	0.501
Nimesulide/morphine	0.512

Interaction index values are listed in ascending order. Lower values indicate higher potency of the combinations.

indicates that the strength of the combinations may depend mainly on COX-2 selectivity (Warner and Mitchell, 2004). However, in previous reports PARE has been shown to be ineffective by the i.v. route in visceral pain models (Padi et al., 2004). In addition, the study of Wong et al. (2000), using the tail-flick test in rats, found that the intrathecal administration of COX-2 inhibitors attenuated the development of tolerance to morphine without directly enhancing its antinociceptive effect; however, it is well recognized that tail-flick and writhing tests measure different types of pain. Even if COX-2 is expressed constitutively in the spinal cord, in rats with neuropathic pain or with an incisional model of postoperative pain, the intrathecal administration of the COX-2 selective inhibitor NS-398 had no effect on spinal cord pain processing (Lashbrook et al., 1999; Zhu et al., 2003). These differences could be due to different animal species and routes of administration (mice vs rats, i.v. vs i.t.), different algesiometric test (tail-flick vs writhing test) or variations in the test methodologies (1% vs 0.6% acetic acid solution, 20 min vs 5 min). Nevertheless, using behavioral studies in preclinal models of nociception, it has been reported that the intrathecal administration of COX-2 inhibitors has significant analgesic activity in hyperalgesic states not associated with inflammation and it is argued that the main antinociceptive mechanism of COX-2 inhibitors lies in the modulation of the constitutive COX-2 present at spinal level (Svensson and Yaksh, 2002). This correlates with the strength of the interactions found in the present work, which agree with the reported selectivity of each NSAID (Warner and Mitchell, 2004). However, since the drugs were injected in the same solution, the possibility of a pharmacokinetic or chemical interaction cannot be excluded.

The presence of constitutive COX-2 in neurons of all lamina of the spinal cord, particularly in the superficial layers (Warner and Mitchell, 2004), gives a rationale for the acute antinociception effect of intrathecal COX-2 inhibitors (Yaksh et al., 2001). The findings of the present work contribute to support the conclusion drawn from other studies, that prostanoids generated by COX-2 at spinal level contribute to the maintenance of hyperalgesia (Samad et al., 2001; Seybold et al., 2003). Furthermore, the distribution and anatomical localization of opioid receptors have demonstrated that the μ-opioid receptor, which is activated by morphine, is highly concentrated in the outer laminae of the dorsal horn of the spinal cord (Ossipov et al., 2004). The present results reinforce the previous suggestion that, in acute pain, a single agent may be less effective than a combination of analgesics with different mechanisms of action (Phillips and Currier, 2004). However, they are not in agreement with the report that parecoxib, an active metabolite of valdecoxib which is a COX-2 selective and specific inhibitor, is not involved in significant interactions with analgesic drugs (Langford, 2002).

The findings of the spinal synergism between morphine and NSAIDs that are preferential or selective inhibitors of COX-2 are concordant with the results obtained previously in several preclinical and clinical studies that emphasize the morphine-sparing effect of NSAIDs, which means that less opioids have to be administered to obtain a given analgesic effect (Reuben and Connelly, 2000; Kroin et al., 2002; Malan et al., 2003).

It has been suggested that in the abdominal constriction pain model of mice, the components of the L-arginine/nitric oxide/cGMP cascade may participate in nociceptive processes both peripherally and centrally by a direct effect on nociceptors or by the involvement of other related pathways of nociceptive processes induced by NO (Abacioglu et al., 2000). NO is involved in the antinociceptive activity of MOR (Przewlocki and Przewlocka, 2001) and the i.t. administration of morphine modulates spinal antinociception by interaction with the NO-glutamate cascade (Watanabe et al., 2003). On the other hand, the activity of COX-2 may be stimulated by NO (Dudhgaonkar et al., 2004), which in turn seems to be modulated by the i.t. administration of MOR. In addition, COX-2 and inducible NO synthase are frequently co-regulated (Simmons et al., 2004). In the present work, the influence of the nitridergic system was not studied, but it could partly explain the synergistic activity of the combination of MOR and COX-2 selective inhibitors.

The control of visceral pain observed in mice by these intrathecal drug combinations may be of clinical relevance in several types of situations. The analgesic effects of MOR in the control of visceral pain are limited and a large amount is generally required. The results demonstrate that the coadministration of COX-2 inhibitors significantly increases MOR-induced antinociception and results in an opioid sparing action which may be useful in the clinical treatment of severe pain. One of the most significant advantages should be the reduction of opioid toxicity which often acts as a major obstacle in pain treatment.

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