Spinal synergy between nonselective cyclooxygenase inhibitors and morphine antinociception in mice

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Abstract

The antinociception induced by the intrathecal coadministration of combinations of morphine with the nonsteroidal anti-inflammatory drugs (NSAIDs) naproxen, piroxicam, metamizol, diclofenac and ketoprofen was studied by isobolographic analysis in the acetic acid writhing test of mice. The effective dose that produced 50% antinociception (ED_{50}) was calculated from the log dose–response curve of intrathecally administered fixed ratio combinations of morphine with each NSAID. By isobolographic analysis, this ED_{50} was compared to the theoretical additive ED_{50} calculated from the ED_{50} of morphine and of each NSAID alone. As shown by isobolograms, all the combinations were synergistic, the experimental ED_{50}'s being significantly smaller than the theoretically calculated ED_{50}'s. The results of this study demonstrate potent interactions between morphine and NSAIDs and validate the clinical use of the combinations of opioids and NSAIDs in pain treatment, even by the intrathecal route.

Theme: Sensory systems
Topic: Pain modulation: pharmacology

Keywords: Antinociception; NSAID; Morphine; Synergism; Writhing test; Isobologram

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are effective drugs for the control of pain. NSAIDs induce antinociception when used intrathecally, either in animal models or in humans [10,24–28,32,35]. The inhibition of cyclooxygenase (COX) enzymes is the main mechanism responsible for both the efficacy and the adverse side effects of NSAIDs. COX-1 isoenzyme is constitutive, and COX-2 is constitutive in certain cells but is also inducible in cells by different inflammation mechanisms; the selectivity of NSAIDs for inhibiting these isoenzymes is different, many drugs are unselective or show preferential inhibition for one of the isoenzymes. Thus, NSAIDs can be ranked according to their COX-1 or COX-2 selectivity [43].

In the analgesic effect of NSAIDs, additional and alternative mechanisms of action have to be considered since the neurotransmission of pain information to the higher centers of the brain is not a passive and simple process. In the dorsal horn of the spinal cord several peptides (i.e., substance P), amino acids (i.e., glutamate, γ-aminobutyric acid) and neurotransmitters (i.e., serotonin, norepinephrine, nitric oxide and arachidonic acid metabolites) are implicated in the transmission and regulation of pain information [17,18,36,38,44]. Thus, depletion of substance P [30]; ATP-sensitive K^+ channels [3,4]; the NO-cGMP-K^+ channel pathway [29]; central opioid receptors [6]; adrenergic [26,32], cholinergic [27] and glutamatergic mechanisms [36]; the NO-cGMP system [13,21]; and systemic and spinal endogenous opioids [14] are involved in the antinociceptive effects of NSAIDs.

Opioids are the most effective and widely used drugs for the treatment of severe pain; however, unwanted side effects

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may seriously limit their clinical use. Opioids can be used intrathecally for postoperative pain control in major surgery [11]. Some combinations of opioids with NSAIDs have synergistic interactions and are in clinical use for postoperative pain [17,33,40,45]. However, there are few reports studying synergy using isobolographic analysis in animal algesiometric models [9,20,23].

The aim of the present work is to further assess the type of interactions of the intrathecal administration of morphine and some NSAIDs which are unselective inhibitors of COX but are stronger inhibitors of COX-1 than of COX-2 (ketoprofen, naproxen, metamizol or dipyrone, piroxicam and diclofenac), evaluated by isobolographic analysis using a chemical algesiometric test.

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 acclimatized to the laboratory for at least 2 h before testing, were used only once during the protocol and were sacrificed immediately after the algesiometric test. The number of animals was kept at a minimum compatible with consistent effects of the drug treatments.

2.2. Intrathecal injections

As previously described [25], 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 and dissolved in a slightly hyperosmotic solution of glucose (6%) to limit rapid diffusion of the drug to higher levels of the spinal cord. The withdrawal of the tail during insertion of the needle is indicative of a successful spinal administration. 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. Mice were injected intraperitoneally (i.p.) 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 maximum 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 in a 5 min period was counted, starting 5 min after acetic acid administration. Antinociceptive activity was expressed as percent inhibition of the usual number of writhes observed in control animals (19.7 ± 0.31, n = 72).

2.4. Protocol

Dose–response curves for morphine (MOR), ketoprofen (KETO), naproxen (NAPRO), metamizol (META), piroxicam (PIRO) and diclofenac (DICLO) were obtained using at least six animals of at least four doses each. A least-squares linear regression analysis of the log dose–response curve allowed the calculation of the dose that produced 50% of antinociception (ED_{50}) for each drug alone. A dose–response curve was also obtained by the coadministration of MOR with each NSAID in combinations of fixed ratios based on fractions of their respective ED_{50} values: 1/2, 1/4, 1/8, 1/16 (ratio values given in Table 2). Isobolographic analysis was used to determine drug interactions. The method has been described previously in detail [24,26–28]. Supra-additivity or synergistic effect is defined as the effect of a drug combination that is higher and statistically different (ED_{50} significantly lower) than the theoretical calculated equieffect 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 [39]. The interaction index was calculated as experimental ED_{50}/theoretical ED_{50} [39]. If the value is close to 1, the interaction is additive. 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 [39].

2.5. Drugs

The following NSAIDs were freshly dissolved in a slightly hyperosmotic solution of glucose (6%) to limit diffusion and were provided by local pharmaceutical companies: diclofenac by Novartis Chile S.A., ketoprofen by Rhone-Poulenc Rorer; metamizol by Sanderson S.A.; naproxen by Laboratorios Saval S.A.; and piroxicam 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 (95% CL). The statistical difference between theoretical and experimental values was assessed by Student's t test for independent means. The program used to perform procedures was Pharm Tools Pro (version 1.27,

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

Drugs	ED_{50} mg/kg i.t. (CL)
Morphine	0.00018 (0.00009–0.00034)
Diclofenac	0.43 (0.41–0.46)
Naproxen	0.48 (0.37–0.62)
Piroxicam	0.51 (0.42–0.62)
Metamizol	0.80 (0.40–1.55)
Ketoprofen	0.86 (0.61–1.11)

Values are ranked in ascending order of potency.

The McCary Group Inc.). P values less than 0.05 ($P < 0.05$) were considered significant.

3. Results

3.1. Antinociception induced by NSAIDs and morphine

The i.t. administration of DICLO, KETO, META, NAPRO, PIRO and MOR produced dose-dependent antinociceptive effects with different potencies in the writhing test of mice. The ED_{50} values and 95% confidence limits (CL) for the antinociceptive effects of morphine and NSAIDs are shown in Table 1. As can be seen, i.t. MOR is more than 3000 times as potent as NSAIDs.

3.2. Interactions between NSAIDs and morphine

The antinociceptive activity of combinations of each NSAID with MOR at fixed ratios of ED_{50} fractions was assessed by an analysis of the dose–response curves obtained after i.t. administration.

The isobolographic analysis of the combinations MOR/NAPRO, MOR/PIRO, MOR/META, MOR/DICLO and MOR/KETO, administered i.t., resulted in a synergistic interaction, as can be see in Fig. 1. Table 2 shows the experimental and the theoretical additive ED_{50} values for the combinations with their 95% CL and the combinations fixed ratios. In addition, the interaction index values of the combinations demonstrated the following rank of potencies for the combinations: MOR/NAPRO >> MOR/PIRO > MOR/META > MOR/DICLO > MOR/KETO (Table 3).

4. Discussion

In agreement with previous reports, in the present work, the intrathecal administration of several NSAIDs or MOR

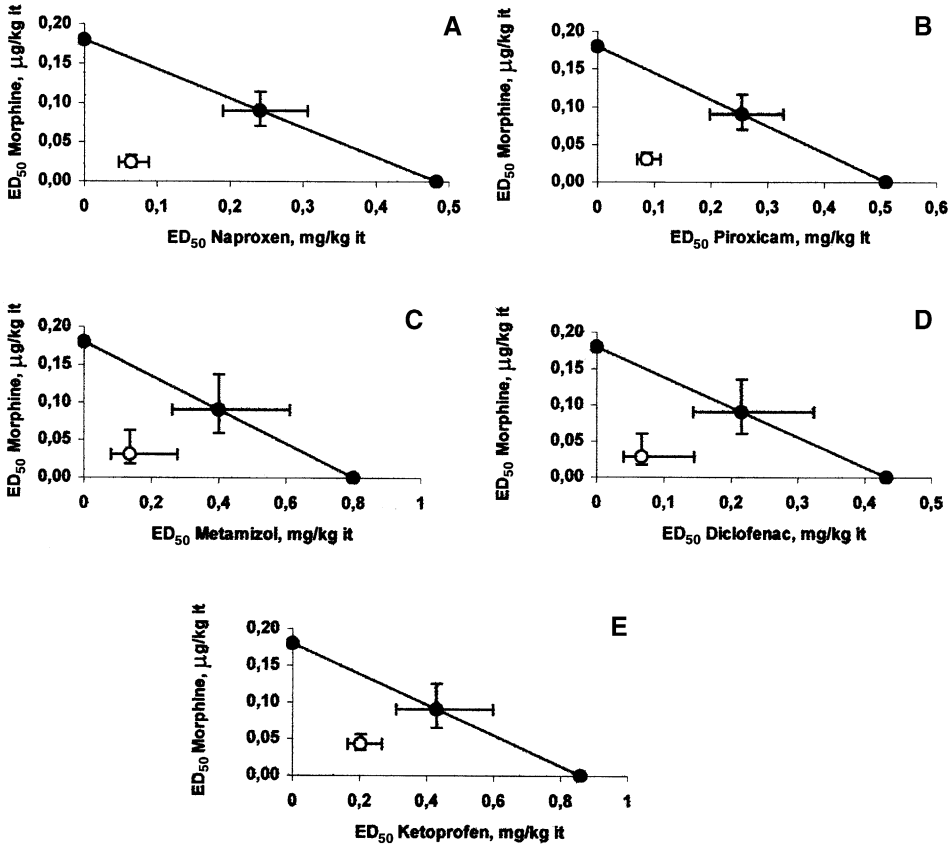

Fig. 1. Isobolograms for the intrathecal administration of the combinations morphine/naproxen (A), morphine/piroxicam (B), morphine/metamizol (C), morphine/diclofenac (D) and morphine/ketoprofen (E). Filled circles correspond to the theoretical ED_{50} with 95% confidence limits, and open circles correspond to the experimental ED_{50} 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 ratios for combinations of NSAIDs with morphine (MOR) administered i.t. in the writhing test of mice

Combinations	ED_{50} values with 95% mg/kg i.t.		Ratio
	Theoretical	Experimental	MOR:NSAID
Diclofenac/Morphine	0.216 (0.17–0.29)	0.095 (0.06–0.2)*	1:2400
Naproxen/Morphine	0.241 (0.20–0.29)	0.088 (0.06–0.12)*	1:2683
Piroxicam/Morphine	0.345 (0.27–0.44)	0.117 (0.09–0.15)*	1:2830
Metamizol/Morphine	0.490 (0.32–0.75)	0.166 (0.09–0.34)*	1:4440
Ketoprofen/Morphine	0.500 (0.37–0.72)	0.244 (0.19–0.32)*	1:4780

* $P < 0.05$ between theoretical and experimental values.

produced analgesia in a tonic pain model, the acetic acid writhing test of mice [27,28,31]. The most important finding is that the combination of nonselective NSAIDs with MOR results in a significant synergy, as demonstrated isobolographically, and that the amount of MOR used to obtain the supra-additivity effect is about 3000 times less than the amount of NSAIDs, as shown by the ratios of the combinations used. In clinical settings, oral combinations of NSAIDs with opioids are used in different situations [33].

The goal of the current study was to examine these interactions when combinations are administered intrathecally, using acute visceral pain paradigm which can be considered a model in clinical relevant intestinal pain in humans [34]. As in previous studies using systemic administration, combinations of MOR and NSAIDs showed very significant synergistic interactions in experimental animals [15,28].

It has been speculated that supra-additive interactions could be ascribed to the activation of complementary pathways of antinociception since the activation of a common mechanism would presumably produce additive effects [27,37]. The main antinociceptive effects of NSAIDs are due to COX inhibition [43], while the effects of opioids are due to the activation of specific opioid receptors, which are linked to several peptides and neurotransmitters [17,19,36,44]. From this point of view, supra-additivity might be an expected result.

The exact mechanism by which NSAIDs could modulate opioid analgesia at spinal or supraspinal sites has not been determined. The synergy obtained in the present work may possibly be due to morphine-induced presynaptic inhibition of the release of excitatory neurotransmitters in the dorsal horn or to less activation of prostanoids receptors found in lamina I and II of the spinal cord, most likely on primary afferent terminals [22]. Centrally, NSAIDs may act on the terminals modulating arachidonic acid pathways involved in opioid activity [8] and, on the other hand, preferential inhibition of COX-1 potentiates the opioid inhibition of GABAergic synaptic transmission in midbrain periaqueductal gray neurons, activating descending antinociceptive pathways and inhibiting spinal nociceptive transmission without spinal interactions between opioids and NSAIDs [41,42]. Thus, the synergistic analgesic effects of NSAIDs and MOR seem to have an important central component.

However, to fully explain the findings obtained in the present work, a possible pharmacokinetic interaction between NSAIDs and opioids cannot be excluded, even if only limited knowledge about the pharmacokinetic interactions between these drugs is available. However, this could be inferred speculating from the data obtained by Ammon et al. [5] that suggest a potential pharmacokinetic interaction of NSAID with the μ-opioid receptors by a noncompetitive inhibition of the major metabolic pathway of opioids. Pharmacokinetic interactions between opioids and NSAIDs have been shown in studies in human liver microsomes in vitro [16].

The activation of the NO-cGMP system may also be involved in the synergy observed in the current experiments. The modulation of spinal antinociceptive activity through this pathway has been described for both types of drugs, and a cooperative effect between NSAIDs and MOR in this respect cannot be ruled out [1,2,7,12,21,29,44].

The potentiation of MOR analgesia by NSAIDs coadministered intrathecally shows the complexity of the interactions seen in this study. However, it remains to be determined if the synergy seen with these combinations in animal studies is the same when the combinations are used clinically. It is possible that this type of study may not predict the clinical usefulness of the combinations. Nevertheless, in a clinical setting, it may be useful to examine different combinations of opioids and NSAIDs to obtain a better individualized pain control and less unwanted side effects. Since the coadministration of NSAIDs and morphine implicates more than one antinociceptive pathway, their use may be of potential aid in the treatment of diverse clinical pain conditions.

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

Combination	Interaction index (I.I.)
Naproxen/morphine	0.268
Piroxicam/morphine	0.339
Metamizol/morphine	0.340
Diclofenac/morphine	0.356
Ketoprofen/morphine	0.471

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

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