Available online at www.sciencedirect.com
SCIENCE DIRECT
Brain Research 1049 (2005) 165 – 170
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research Report

Spinal synergy between nonselective cyclooxygenase inhibitors and morphine antinociception in mice

Hugo F. Miranda*, Juan Carlos Prieto, Gianni Pinardi

Phramacology Program, ICBM, Faculty of Medicine, University of Chile, PO Box 70.000, Santiago 7, Chile

Accepted 22 April 2005
Available online 3 June 2005

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

* Corresponding author. Fax: +56 2 737 2783.
E-mail address: hmiranda@med.uchile.cl (H.F. Miranda).

0006-8993/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.brainres.2005.04.068

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 post-operative 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,