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Isobolographic analysis of multimodal analgesia in an animal model of visceral acute pain

Hugo F. Miranda [a], Juan Carlos Prieto [a,b], Margarita M. Puig [c], Gianni Pinardi [a,*]

[a] *Pharmacology Program, ICBM, Faculty of Medicine, University of Chile, Clasificador 70.000, Santiago 7, Chile*
[b] *Cardiovascular Center, Hospital Clínico University of Chile, Chile*
[c] *Department of Anesthesiology, Hospital del Mar, UAB, Paseo Marítimo 25, 08003 Barcelona, Spain*

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Abstract

Multiple analgesic–drug combinations are commonly used in the management of acute and chronic pain in humans during multimodal or balanced analgesia. At present, these combinations are used empirically in clinical practice and are considered to be beneficial for the patient. Interactions between two antinociceptive drugs have been thoroughly examined, but the nature of interactions between three analgesics has not been studied. The antinociceptive interaction of ketoprofen (K) with a mixture of morphine (M) and paracetamol (P) was evaluated using a model of visceral acute tonic pain, the acetic-acid writhing test in mice. The i.p. administration of the combination M/P+K resulted in a significant potentiation of the antinociception induced either by K or by the synergic two-drug mixtures M/K, P/K and M/P. The effect of opioid, cholinergic, adrenergic and serotonergic antagonists on the analgesic interaction was assessed. The pretreatment of mice with atropine (1 mg/kg) did not produce any change in the synergistic interaction of the triple combination. The pretreatment with naltrexone (1 mg/kg) or tropisetron (1 mg/kg) reduced the intensity of M/P+K synergic interaction, while prazosin (0.1 mg/kg) significantly potentiated the synergy. The findings of this work suggest that the two major pathways of descending inhibitory systems, noradrenergic and serotonergic are significantly involved in the mechanism of the antinociceptive synergy induced by the triple combination. On the other hand, opioid pathways also seem to participate, since pretreatment with naltrexone reduced the synergy. In conclusion, the triple combination M/P+K induced a strong synergistic antinociceptive effect, which could be of interest for optimal multimodal clinical analgesia.

Keywords: Ketoprofen; Morphine; Paracetamol; Antinociception; Writhing test; Isobologram; Synergy

1. Introduction

Pain is the net effect of multidimensional mechanisms that involve most parts of the central nervous system (Le Bars et al., 2001) and its treatment is probably one of the major challenges in clinical medicine. According to this view, a variety of drugs, alone or in combination, have been tested in different preclinical models of pain with variable results, depending on the models and tests used. For instance, opioids show good antinociceptive effects in models of thermal stimulation, where non-steroidal anti-inflammatory drugs (NSAIDs) produce inconsistent results. Several drug combinations which have synergistic analgesic interactions have been tested preclinically and clinically (Elia et al., 2005). For example, it has been reported that gabapentin, the (*S*)-ketoprofen isomer, and the cannabinoid agonist CP 55,940, enhance the analgesic effect of morphine (Ossipov et al., 2000; Pakulska and Czarnecha., 2004; Tham et al., 2005). Combinations of NSAIDs and adrenergic drugs are also synergic (Miranda et al., 2001; Pinardi et al., 2001). Moreover, it has been recently reported that NSAIDs and morphine show a synergistic interaction (Miranda et al., 2004, 2005; Pinardi et al., 2005). At present, interactions between two analgesic drugs have been thoroughly examined, but to date, the nature of interactions between three analgesic drugs has not been studied extensively. However, multiple analgesic–drug combinations (mainly an NSAID+paracetamol+an opioid) are commonly used in clinical practice to manage acute and chronic pain syndromes (Elia et al., 2005; Skinner and Shintani, 2004). The aim of the present work was to experimentally evaluate the antinociceptive

* Corresponding author. Tel.: +56 2 678 6237; fax: +56 2 737 2783.
E-mail address: gpinardi@med.uchile.cl (G. Pinardi).

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interaction of ketoprofen with the clinically frequently used combination of morphine and paracetamol (M/P), using a model of visceral acute tonic pain, the acetic acid-induced writhing test of mice. In this test, the combination M/P has shown a strong supra-additive interaction (Miranda et al., 2004, 2005). Furthermore, attempting to clarify the mechanisms of the interaction, the effect of opioid, cholinergic, adrenergic and serotonergic system pathways, usually involved in analgesia, was assessed by the use of specific antagonists. This investigation attempts to use isobolographic analysis to examine a triple combination of analgesic drugs.

2. Materials and methods

2.1. Animals

Male CF-1 mice (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 sacrified immediately after the algesiometric test. The number of animals was kept at a minimum compatible with consistent effects of the drug treatments (6-8 mice per experimental group).

2.2. Writhing test

The procedure used has been described previously (Miranda et al., 2001). Briefly, mice were injected intraperitoneally (i.p.) with 10 mL/kg of 0.6% acetic acid solution, 30 min after the i.p. administration of the drugs, a time at which preliminary experiments showed to be the correct time interval for the maximal effect of all drugs used. Each mouse was then placed in an individual clear plexiglass observation cylinder (20×20 cm). A writhe was defined as 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 the acetic acid administration. Antinociception was expressed as percent inhibition of the number of writhes observed in saline control animals (19.7±0.27, n=22).

2.3. Protocol

Individual dose–response curves for paracetamol (P), morphine (M) and ketoprofen (K) were obtained using at least six animals per dose and at least four doses. A least-squares linear regression analysis of the log dose–response curve allowed the calculation of the dose that produced 50% of control writhes (ED_{50}) when each drug was administered alone. Dose–response curves were also obtained and analyzed for the mixtures of morphine plus ketoprofen (M/K), paracetamol plus ketoprofen (P/K) and morphine plus paracetamol (M/P), administered in fixed ratio combinations based on fractions (1/2, 1/4, 1/8, 1/16) of the ED_{50} of each drug in the combination (Pinardi et al., 2003). Afterwards, the calculated ED_{50} of the dose–response curve originated from the M/P mixture, was combined with the ED_{50} of K (Table 1) and co-administered to mice in the same fractions as above. The new dose–response curve (three-drug combination) was then analyzed to obtain the ED_{50} of the triple combination. With the ED_{50}'s of K and M/P, an isobolographic analysis was performed to characterize the interaction, treating M/P as a single drug and comparing the isobologram to the one calculated for the M/P combination.

Dose–response curves for the three-drug combination were also obtained after the animals were pretreated with 1 mg/kg of i.p. atropine, tropisetron, naltrexone or 0.1 mg/kg of i.p. prazosin. The antagonists doses were selected from literature references (Pinardi et al., 1998; Miranda et al., 2004; Giordano and Gerstmann, 2004; McQueen et al., 2007). A similar isobolographic analysis was used to characterize the drug interactions after these pretreatments.

A detailed description of the isobolographic analysis has been previously published (Miranda et al., 2002, 2004; Pinardi et al., 2005). Supra-additivity or synergy is defined as the effect of a drug combination that is higher and statistically different (ED_{50} significantly lower) than the theoretically calculated equieffect of drugs combined in the same proportions. If the ED_{50}'s are not statistically different, the effect of the combination is additive, meaning that each constituent contributes with its own potency to the total analgesic effect (Tallarida, 2001). The interaction index was calculated as the ratio between the experimental ED_{50}/the theoretical ED_{50}.When this value is close to 1, there is no interaction and the final effect 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 (Tallarida, 2001).

2.4. Drugs

All drugs were freshly dissolved in saline. Ketoprofen was provided by Rhone-Poulenc Rohrer, Chile; paracetamol by Bristol–Myers–Squibb, France; tropisetron hydrochloride by Novartis Chile S.A.; atropine sulfate, morphine sulfate,

Table 1
ED_{50} values±SEM for the antinociceptive effect of i. p. morphine, paracetamol, ketoprofen and the combinations M/K, P/K, M/P and M/P+K in the writhing test of mice

	ED_{50}±SEM (mg/kg)	Combinations drug ratio
Morphine	0.12±0.011	
Paracetamol	49.46±3.32	
Ketoprofen	30.30±3.85	
M/K	3.98±0.22	1: 0.004
P/K	20.37±1.12	1: 1.63
M/P	10.11±0.9	1: 0.003
M/P+K	3.73±0.21	1: 0.33

M/K: combination of the ED_{50} of morphine+the ED_{50} of ketoprofen.
P/K: combination of the ED_{50} of paracetamol+the ED_{50} of ketoprofen.
M/P: combination of the ED_{50} of morphine+the ED_{50} of paracetamol.
M/P+K: combination of the ED_{50} of M/P+the ED_{50} of ketoprofen.