Pharmacological Reports
2013, 65, 80–88
ISSN 1734-1140

Systemic synergism between codeine and morphine in three pain models in mice

Hugo F. Miranda[1,2], Viviana Noriega[2], Ramiro J. Zepeda[1], Fernando Sierralta[3], Juan C. Prieto[1,4]

[1]Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Medical School, University of Chile, Santiago, Chile

[2]Medical School, Pharmacy School, Universidad Andrés Bello, Santiago, Chile

[3]Dental School, Universidad Finis Terrae, Santiago, Chile

[4]Cardiovascular Department, Clinical Hospital, University of Chile, Santiago, Chile

Correspondence: Hugo F. Miranda, e-mail: hmiranda@med.uchile.cl

Abstract:
Background: The combination of two analgesic agents offers advantages in pain treatment. Codeine and morphine analgesia is due to activation of opioid receptor subtypes.
Methods: This study, performed in mice using isobolographic analysis, evaluated the type of interaction in intraperitoneal (*ip*) or intrathecal (*it*) coadministration of codeine and morphine, in three nociceptive behavioral models.
Results: Intrathecal morphine resulted to be 7.5 times more potent than *ip* morphine in the writhing test, 55.6 times in the tail flick test and 1.7 times in phase II of the orofacial formalin test; however, in phase I of the same test *ip* was 1.2 times more potent than *it* morphine. Intrathecal codeine resulted being 3.4 times more potent than *ip* codeine in the writhing test, 1.6 times in the tail flick test, 2.5 times in phase I and 6.7 times in phase II of the orofacial formalin test. Opioid coadministration had a synergistic effect in the acute tonic pain (acetic acid writhing test), acute phasic pain (tail flick test) and inflammatory pain (orofacial formalin test). The interaction index ranged between 0.284 (writhing *ip*) and 0.440 (orofacial formalin phase II *ip*).
Conclusion: This synergy may relate to the different pathways of pain transmission and to the different intracellular signal transduction. The present findings also raise the possibility of potential clinical advantages in combining opioids in pain management.

Key words:
opioids, algesiometer tests, isobolographic analysis, synergism

Abbreviations: DOR – delta opioid receptor, ED_{50} – dosage that produced 50% of MPE, I.I. – interaction index, *ip* – intraperitoneal, *it* – intrathecal, KOR – κ opioid receptor, MPE – maximum possible effect, MOR – μ opioid receptor, NSAIDs – nonsteroidal anti-inflammatory drugs, NOP – nociceptin opioid receptor, SEM – standard mean error

Introduction

Opioids are frequently used in the treatment of moderate to severe pain. Opioids mimic the action of endogenous opioid peptides by interacting with opioid recep-

tor subtypes. Four opioid receptors have been cloned and are referred to as MOR, DOR, KOR, and NOR receptors, for mu (μ), delta (δ), kappa (κ), and nociceptin receptors, respectively [29]. Multimodal analgesia, which is the combination of analgesic agents, offers important benefits in the management of both acute and chronic pain. The mixture of different analgesic agents can achieve improved efficacy and/or tolerability and safety compared to equianalgesic doses of the individual drugs. Combining different agents also enhances efficacy in complex pain conditions involving multiple causes [22]. Interactions between opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) have been previously reported [10, 11].

Furthermore, it has been communicated that coadministration of opioids induced a potent analgesic synergy in a mechanical nociceptive assay [9, 24]. Moreover, a marked antinociceptive synergy has been demonstrated by the coadministration of oxycodone and morphine [20]. When L-methadone was associated with several μ opioid ligands, a synergistic effect was observed. Out of the compounds examined, L-methadone selectively synergizes with morphine, morphine-6-glucuronide, codeine, and the active metabolite of heroin, 6-acetylmorphine [28]. On the other hand, since codeine is a morphine prodrug and only 5% of the dose is O-demethylated to morphine, it is suggested that codeine analgesia does not depend on morphine formation [28, 29]. Pharmacokinetic features of codeine and the relative scarcity of behavioral model studies to evaluate the interaction between morphine and codeine justify this preclinical study. Furthermore, there have been clinical reports about differences in analgesic efficacy among MOR opioid agonists.

Considering the background mentioned above, the purpose of this study was to examine the analgesic interaction between morphine and codeine as not all opioid MOR agonists demonstrate synergism when administered in combination [2]. In this study we used intraperitoneal (*ip*) or intrathecal (*it*) coadministration of codeine and morphine in three animal nociceptive behavioral models. The evaluation of interaction was performed through isobolographic analysis.

Materials and Methods

Animals

Male CF-1 mice (30 g), housed on a 12 h light-dark cycle at 22 ± 2°C with *ad libitum* access to food and water were used. Experiments were performed according to 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 University of Chile Medical School. Animals that 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 test. The number of animals was kept at a minimum compatible with consistent effects of the drug treatments. All assays were conducted by an experimented observer who was unaware of the drug treatment of each individual mouse.

Dose-response curves for administration of codeine (1, 3, 10 and 30 mg/kg, *via ip* and *it*) and morphine (0.01, 0.03, 1, and 3 mg/kg *via ip* and the same doses *via it*) were obtained using at least six animals for each of at least four doses, 30 min after drug application. A least-square linear regression analysis of the log dose-response curve allowed the calculation of the doses that produced 50% (ED_{50}) antinociception when each drug was administered alone (Tab. 1).

Tab. 1. ED_{50} values ± SEM (mg/kg) for the antinociceptive effect of codeine and morphine administration *ip* and *it* in the writhing, tail flick and orofacial formalin tests in mice

Test	Codeine *ip*	Codeine *it*	Morphine *ip*	Morphine *it*
Writhing	6.17 ± 0.92	1.84 ± 0.21*	0.12 ± 0.01	0.016 ± 0.003◆
Tail flick	46.95 ± 4.42	29.99 ± 2.24*	5.01 ± 0.92	0.09 ± 0.003◆
Orofacial formalin Phase I	5.76 ± 0.61	2.33 ± 0.84*	0.17 ± 0.02	0.21 ± 0.02
Orofacial formalin Phase II	9.33 ± 0.67	1.40 ± 0.45*	0.34 ± 0.02	0.19 ± 0.06◆

* $p < 0.05$ compared with codeine *ip*; ◆ $p < 0.05$ compared with morphine *ip* (Student's *t*-test)