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Antinociceptive synergy between diclofenac and morphine after local injection into the inflamed site

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Abstract:
Background: Combinations of non-steroidal anti-inflammatory drugs with opioids are frequently used to reduce opioid doses required in the clinical management of acute pain. The present study was designed to evaluate the possible antinociceptive interaction between morphine and diclofenac at peripheral level in male rats.
Methods: Drugs were chosen based on their efficacy in the treatment of this kind of pain and as representative drugs of their respective analgesic groups. For the formalin test, 50 µl of 1% formalin solution was injected subcutaneously into the right hind paw. The interaction between morphine and diclofenac was evaluated by using isobolographic analysis and interaction index. Drug interaction was examined by administering fixed-ratio combinations of morphine-diclofenac (1 : 1 and 3 : 1) of their respective ED_{30} fractions.
Results: Diclofenac and morphine reduced flinching behavior in a dose-dependent manner during phase 2 but not phase 1 of the formalin test. Isobolographic analysis showed a synergistic interaction for the combination of morphine and diclofenac after local peripheral administration.
Conclusions: Data suggest that the combination of morphine with diclofenac at the site of injury is synergistic and could be useful in the treatment of wounds, bruises, rheumatisms and other painful peripheral conditions associated with an inflammatory process.

Key words:
antinociception, diclofenac, morphine, isobolographic analysis, formalin test

Introduction

Inflammatory pain is characterized by spontaneous pain and hypersensitivity to both noxious and non-noxious stimuli. After tissue injury, diverse inflammatory mediators are released in the periphery, including prostaglandins (PGs). In turn, synthesized PGs at the site of injury play an important role in the peripheral sensitization of nociceptors to stimuli. The inhibition of cyclooxygenase (COX), a key enzyme in PGs synthesis, is the principal target of the non-steroidal anti-inflammatory drugs (NSAIDs). Therefore, local peripheral application of NSAIDs is an alternative to produce antinociception.

On the other hand, besides their central mechanisms of action, opioids also produce antinociception through peripheral mechanisms which involve opioid receptors [44]. The activation of peripheral opioid receptors has been shown to induce antinociception in a variety of models, particularly in inflammatory conditions [2, 39, 44] including the formalin test [4, 10, 15, 28, 49]. Like NSAIDs, opioids offer an alternative to produce antinociception *via* direct application into injured peripheral tissue [30, 56].

Diclofenac is a non-selective COX inhibitor [23, 27] and it is considered as one of the most effective NSAIDs for local peripheral use in clinical [5, 41, 43, 51] and preclinical [16, 50] comparative studies. In addition, it is often used as reference drug to compare other NSAIDs and new analgesic drugs in inflammatory pain [7, 19, 38]. However, local application of diclofenac can produce adverse effects as dermatitis, skin rash, dry skin, desquamation and pruritus, among others [22]. Mechanistically, diclofenac has shown peripheral effects that are not mediated by COX. In fact, it has already been suggested that in addition to the inhibition of COX, this drug could activate the nitric oxide-cyclic GMP-potassium channels (NO-cyclic GMP-K^+ channels) pathway [32, 48].

There are convincing evidence that morphine has also the capacity to reduce nociception by local application in animals [6, 53] and human beings [11, 17, 35] in an effective and safe manner. This drug not only antagonize opioid receptors, but also activates the peripheral NO-cyclic GMP-K^+ channels pathway [39] suggesting a possible interaction with diclofenac at the local peripheral level through different mechanisms and/or by a shared pathway.

Analgesics combinations constitute the basis for multimodal or balanced analgesia. In clinical practice, NSAIDs are combined with morphine to relieve postoperative pain [21]. In this sense, clinical studies have described a 30–50% reduction in the opioid requirement with added NSAIDs [21]. In addition to clinical studies, combinations of NSAIDs with morphine have been evaluated in experimental pain models after systemic [24–26, 34] and local peripheral administration [20]. Other opiates such tramadol [40], codeine, nalbuphine [18, 31], have been combined with NSAIDs. However, to the best of our knowledge, there are not studies assessing the diclofenac/morphine combination applied directly into inflamed peripheral tissue. In this regard, the objective of the current study was to evaluate the combination of diclofenac and morphine injected into the inflamed paw in the rat formalin test.

Materials and Methods

Animals

Experiments were performed on adult male Wistar rats (body weight range 180–200 g), 6–8 weeks of age. Animals were obtained from our own breeding facilities and were housed and maintained at 22 ± 0.5°C under 12-h light/12-h dark cycle. Food and water were available *ad libitum*. The experiments were carried out during the light phase from 10:00 a.m. to 16:00 p.m. The study protocol was approved by the local Ethics Committee of our Institution, in accordance with the International Association for the Study of Pain guidelines on ethical standards for investigations of experimental pain in conscious animals [55]. Furthermore, every effort was done to minimize pain and suffering in animals and the number of rats used was the minimal required to obtain significant statistical power.

Drugs

Diclofenac sodium and morphine sulfate were purchased from Merck (Mexico City) and PISA (Mexico City), respectively. The drug solutions were freshly prepared in saline (0.9% w/v NaCl).

Injections

Drugs, either alone or as a mixture, were injected subcutaneously (*sc*) in a volume of 50 µl into the dorsal surface of the right hind paw. Each rat received 2 injections, one of vehicle, diclofenac, morphine or the diclofenac-morphine mixture and another of 1% formalin. Appropriate controls for the injection and vehicles were performed before starting the formal study. In addition, a control rat for the vehicle was tested each experimental day together with the drug treated groups.

Formalin-induced nociception test

On testing day, rats were acclimated to individual open acrylic observation chambers until explorative behavior ceased (approximately 30 min), in order to minimize stress. The formalin test was carried out as previously described [54]. In brief, 50 µl of diluted formalin (1%) was injected subcutaneously into the dorsal region of the right hind paw of the rats, with a 30-gauge needle. Following the formalin injection, the animals were returned to the observation chambers and the pain-related behavior was quantified by counting the incidence of spontaneous flinching, which was defined as rapid and brief withdrawal, or as flexing of the injected paw, during 1-min periods every 5 min for 60 min after injection. Data collected