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Pharmacological profile of dexketoprofen in orofacial pain

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ABSTRACT

Background: Non-steroidal anti-inflammatory drugs (NSAIDs) may act through others mechanisms, in addition to inhibition of prostaglandin synthesis. These includes cholinergic, NO, serotonergic and opioids pathways.
Methods: The aim of this work was to evaluate the effect of systemic action of (S)-+-ketoprofen (dexketoprofen, DEX) on pain behaviors using the orofacial formalin test in mice and the potential involvement of cholinergic, NO, serotonergic and opioids pathways.
Results: The pretreatment of the mice with 1 mg/kg *ip* of atropine or opoid antagonists: 1 mg/kg, *ip* of NTX or 1 mg/kg *ip* of NTI or 1 mg/kg of NOR-BNI *ip*, did not produce significant change in the ED_{50} values of the antinociception to orofacial test induced by DEX. The pretreatment of the mice with 0.5 mg/kg *ip* tropisetron, increased in a significant fashion the values of ED_{50} of DEX. When the mice were treated with 5 mg/kg *ip* of L-NAME or 25 mg/kg *ip* of aminoguanidine or 50 mg/kg *ip* of 7-nitroindazole reversed the antinociception of DEX.
Conclusion: The findings of this study demonstrate activation of NO and 5-HTpathways play important roles in the systemic antinociceptive effect of DEX in a murine model of inflammatory pain.

Introduction

In the treatment of pain, either neuropathic or nociceptive, different agents are used, including nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs acts primarily by inhibiting prostaglandin synthesis restraining prostaglandin synthesis acting as inhibitors of cyclooxygenase enzymes (COXs), however its effects can not be explained exclusively by this enzyme inhibition. The effect of NSAIDs on the inhibition of COX, an enzyme that converts arachidonic acid to prostaglandins (PGs), provides an explanation for most of their pharmacological actions, including their anti-inflammatory, analgesic, antipyretic and platelet anti-aggregant effects, as well as for their deleterious side effects, such as stomach ulcer and renal insufficiency. However, several lines of evidence suggest that NSAIDs have additional mechanisms between them appear the ability of NSAIDs to penetrate biological membranes where they disrupt important processes of cellular function [1–5].

The different members of the NSAID family, include among them the derivatives of propionic acid, such as ibuprofen, naproxen, ketoprofen, etc. The most of them have a chiral center where the dextrorotatory enantiomer with S(+) configuration possesses high COX inhibitory activity, in contrast, R(−) enantiomer have poor inhibitory activity. (S)-+-ketoprofen, named, dexketoprofen (DEX), the active dextrorotatory enantiomer of the NSAID ketoprofen has been proved to be effective for relief pain either man and animal models [6–13]. Likewise other NSAIDs, antipyretic, anti-inflammatory, and analgesic mechanisms of action of DEX are based on the inhibition of prostaglandin synthesis. However, the pharmacological profile of DEX suggests that this drug may act through by others mechanisms, which it has been are scarcely investigated.

The present study was designed to evaluate the involvement of the cholinergic, NO, serotonergic and opioids pathways in the antinociceptive effect of DEX in the orofacial formalin inflammatory assay.

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Materials and methods

CF-1 male mice, weighing 28–30 g, housed in a 12-h light-dark at 22° ± 1° C, with free acces to food and water, were used. The animals were acclimatized to the laboratory environment for at least 2 h before use. Experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals issued by the National Institute of Health, and experimental procedures were approved by the Institutional Animal Care and Use Committee. Each animal was used only once, and received only one dose of the drugs tested. All drugs were freshly prepared by dissolving them in normal saline, and administered intraperitoneally (*ip*) and the behavior test was performed by investigators blinded to the treatment groups. Control saline animals were run interspersed concurrently with the drug-treated animals (at least two mice per group), which prevented all the controls being run on a single group of mice at one time during the course of the research.

Orofacial formalin test

To perform the test, the method described previously was used [14]. 20 μL of 2% formalin solution was injected into the upper lip, just lateral to the nose with a 27-gage needle attached to a 50-μL Hamilton syringe. The applied chemical stimulus (formalin) can be considered noxious since it produces tissue injury, activates Aδ and C nociceptors as well as trigeminal and spinal nociceptive neurons. Each mouse was immediately returned to a plexiglass observation chamber. The test shows two clear-cut phases: Phase I corresponds to the 5-min period starting immediately after the formalin injection and represents a tonic acute pain due to peripheral nociceptor sensitization. Phase II was recorded as the 10-min period starting 20 min after the formalin injection and represents inflammatory pain. The nociceptive score was determined for each phase by measuring the total number of seconds that the animals spent grooming the injected area with the ipsilateral extremity. Grooming time for saline control was 103.07 ± 4.68 (n = 20) and 133.20 ± 4.36 (n = 20) seconds, respectively. Drugs or saline were administered to animals 30 min before formalin injection, a time at which preliminary experiments showed occurrence of the maximum effect. Total grooming time in each period was converted to per cent MPE as follows:

%MPE = 100-(postdrug grooming time/control grooming time saline) × 100

The dose that produced 50% of MPE (ED_{50}) was calculated from the linear regression analysis of a dose–response curve obtained by plotting log doses versus % MPE.

Protocol

Dose–response curves for *ip* administration of DEX, before and after pretreatment with different antagonists, were obtained using at least six to eight animals at each of 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 antinociception for DEX.

Drugs

Drugs used in this study were disolved in normal saline (0.9% w/v NaCl) in a constant volumen of 10 ml/kg and *ip* administered as mg/kg on the basis of the salts, included dexketoprofen DEX trometamol was provided by Menarini, Spain. 7-Nitroindazole sodium salt, aminoguanidine hydrochloride, atropine, sulfate, L-NAME (Nω-nitro-L-arginine methyl ester), naltrexone (NTX) hydrochloride, naltrindole (NTI) hydrochloride, nor-binaltorphimine dihydrochloride (NOR-BNI), and tropisetron hydrochloride were purchased from Sigma-Aldrich Chemical Co, St. Louis, MO, USA.

Statistical analysis

Results are presented as mean ± standard error of the mean (SEM) of 6–8 animals per group. All calculations were performed with the program Pharm Tools Pro (version 1.27; McCary Group Inc., PA, USA). p Values under 0.05 ($p < 0.05$) were considered significant.

Results

All experiments performed were carried out using dose of each drug that did not cause any detectable modification in gross behavior, motor coordination, or spontaneous motility activity.

Antinociception induced by DEX orofacial formalin inflammatory pain

The *ip* administration of DEX at doses of 3, 10, 30, 100 and 300 mg/kg displayed a dose dependent antinociception in the phase I and phase II of the orofacial formalin test, as can be seen in Fig. 1. The ED_{50} of DEX in phase I resulted to be 16.66 ± 1.71 mg/kg and in phase II was 50.73 ± 4.04 mg/kg, see Table 1. DEX was 3.04 fold more potent at phase I than phase II.

Interaction of atropine in DEX induced antinociception

The pretreatment of the mice with 1 mg/kg *ip* of atropine, 30 minutes prior to the administration of DEX, lack of effect, in both phases, of the orofacial formalin test, in the antinociception produced by all the doses of the DEX tested. The corresponding ED_{50}'s values are not significant different, compared with the control value, see Table 1.

Interaction of opioidergic agent in DEX induced antinociception

After pretreatment of the mice with 1 mg/kg, *ip* of NTX did not produce significant change in the ED_{50} values of the antinociception to orofacial test induced by DEX, comparing with the mice in the control group. Pretreatment with the opioid antagonists NTI (1 mg/kg *ip*) or NOR-BNI (1 mg/kg *ip*) showed no change in the antinociceptive effect of DEX (see Table 1).

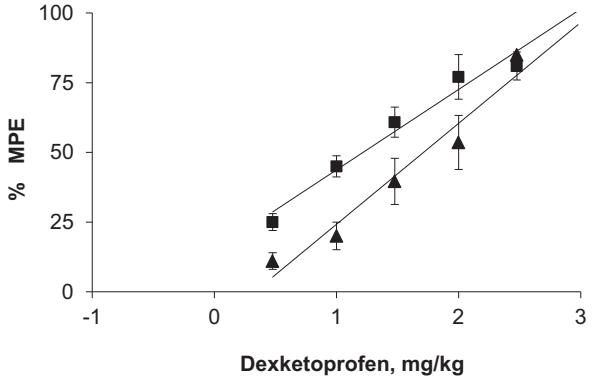

Fig. 1. Dose-response curves for the antinociceptive activity induced by deexketoprofen *via ip* in the fomalin orofacial assay in mice. ▲ = Phase I, ■ = Phase II. Each point is the mean ± SEM of 8 animals. % MPE = antinociception as a percentage of the maximun possible effect.

Table 1
ED_{50} values, in mg/kg ± SEM of the antinociception induced by dexketoprofen (DEX) in phase I and phase II in the presence of cholinergic, opioidergic, serotonergic and nitridergic receptor agents at the orofacial test of mice.

Control	Dose	Fase I- -ED50	Fase II- -ED50
DEX		16.66 ± 1.71	50.73 ± 4.04
Atropine	1 mg/kg	15.07 ± 1.21	49.39 ± 2.52
NTX	1 mg/kg	15.62 ± 1.40	51.41 ± 2.45
NTI	1 mg/kg	18.72 ± 0.60	50.05 ± 1.10
Nor-BNI	1 mg/kg	18.18 ± 2.8	49.68 ± 1.22
Tropisetron	0.5 mg/kg	21.48 ± 0.91 *	56.37 ± 1.16 *
Aminoguanidine	25 mg/kg	24.08 ± 0.78 *	63.06 ± 3.63 *
L-NAME	5 mg/kg	24.96 ± 1.30 *	59.96 ± 2.50 *
7-Nitroindazole	50 mg/kg	22.62 ± 0.98 *	62.11 ± 1.10 *

DEX = dexketoprofen, NTX = naltrexone, NTI = naltrindole, Nor-BNI = norbinaltorphimine, N = 6–8 mice; $p < 0.05$, Student t test.

Interaction of nitridergic agents in DEX induced antinociception

When the mice were pretreated with 25 mg/kg of aminoguanidine, *ip*, the DEX antinociception produced in both phases of the orofacial formalin assay was reversed, since their ED_{50}'s values in both phases were significant different, compared with the control values, as can be seen in Table 1. After pretreatment of the mice with 3 mg/kg *ip* of L-NAME a significant reduction of the effect of DEX was obtained, since the corresponding ED_{50} values in both phase were increased, in comparison with the control values, as can be seen in Table 1. Similarly, pretreatment of the mice with 50 mg/kg, *ip* of 7-nitroindazole, resulted in a significant increase of the ED_{50} values of DEX compared with control mice, see Table 1.

Interaction of serotonergic agent in DEX induced antinociception

The pretreatment of the mice with 0.1 mg/kg *ip* tropisetron, increased in a significant fashion the values of ED_{50} of DEX, compared with the control values, in the orofacial assay, as can be seen in Table 1.

Discussion

Ketoprofen exists as two enantiomers, named S(+) and R(−) enantiomer. These two optically compounds demonstrate different pharmacological actions, so S(+) possesses high COX inhibitory activity and in contrast, R(−) enantiomer have poor inhibitory activity. In this study, was used the S(+) enantiomer,called deketoprofen (DEX) which exhibited a marked antinociceptive activity to formalin orofacial pain characterized as biphasic dose dependent response, which is concordant with previous behavioural assays [6–13]. The lack of parallelism of dose–response curves obtained with DEX, might be secondary to a different mechanism of action of the drug in each phase of the algesiometer assay [16]. Regarding the nature of mechanisms of analgesia, it has been reported that cholinergic mechanisms are involved in analgesia [17], however, the present study demonstrated that the antinociception induced by DEX is not due to an interaction related to an increase in ACh release acting on muscarinic receptor since the induced DEX analgesic ED_{50} was not modified by atropine.

To explore the possible antinociceptive mechanisms of DEX, the role of the opioid receptors was investigated in the orofacial formalin-induced nociception in mice. It is now well known that activation of opioid receptors produces marked analgesia mediated through a combined presynaptic and postsynaptic effect. [18]. In the present study, naltrexone, a non selective opioid antagonist, naltrindole, a DOP antagonist and Nor-BNI, a KOR antagonist were not able to significantly modify the magnitude of DEX antinociceptive effects in the test used in the present work. This results are in agreement with a previous report similar [19] and indicates that DEX-induced antinociception is not mediated through endogenous analgesic opioid system.

In relation to the role of 5-HT in nociception, it has been suggested 5-HT modulates the antinociception, thus 5-HT and 5-HT agonists, raise the nociceptive threshold, while 5-HT antagonists attenuate analgesia [20]. It has been proposed that the action of the 5-HT_3 receptor antagonists can be attributed to an antinociceptive effect that occurs at the same time as an antiphlogistic and probably also an immunosuppressive effect. Whereas an inhibited release of substance P from the nociceptors, and possibly some other neurokins as well, seems to be the most likely explanation for the antinociceptive action, the antiphlogistic effect is primarily due to an inhibited formation of various different phlogistic substances [21]. Also it has been reported that tropisetron may have an analgesic effect of its own [22].

The results obtained in the present study are in line with those that reported that 5-HT_3 receptor antagonist, tropisetron reversed totally the antinociception induced by 5-HT [23] and significantly inhibited the S-(+)-ketoprofen-induced antinociceptive effect [24]. The increase of antinociceptive ED_{50} of DEX induced by pretreatment with tropisetron suggest that 5-HT play important role in the systemic antinociceptive effect of DEX in the formalin orofacial model of mice pain.

Nitric oxide (NO), the smallest signalling molecule known, is produced by three isoforms of NO synthase referred as as neuronal n-NOS (or NOS I), inducible i-NOS (or NOS II), and endothelial e-NOS (or NOS III). [25]. NO is involved in many physiological processes and several lines of evidence have indicated that NO plays a complex and diverse role in the modulation of pain [25,26]. In the present study, after pretreatment with L-NAME, the ED_{50} of DEX was significantly increased compared with control mice in the orofocial formalin induced pain assay suggesting that NO is involved in DEX antinociception. A similar change in pain response was found when the pretreatment of the mice with the i-NOS inhibitor aminoguanidine or the n-NOS inhibitor 7-nitroindazole. These results indicate that DEX produced antinociception in the orofacial formalin test through mechanisms that involve an interaction with NO system, since inhibition of NOS could modulate inflammatory orofacial antinociception by regulating citokyne expression [27]. The results obtained with DEX and drugs related to NO inhibition are not in agreement to those reported previously [24] the explanation of these difference could be related to the different protocol used (rats *vs.* mice; route of administration*: ip vs. it or icv*; doses: μg *vs.* mg).

In conclusion, this study utilized a mice model of formalin orof, serotonergic and opioids pathways in the systemic action of DEX. Results show that activation of NO and 5-HT pathways play important roles in the systemic antinociceptive effect of DEX in a murine model of inflammatory pain.

Conflict of interest

The authors declare that they have no conflict of interest.

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