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Noradrenergic modulation of Dex-ketoprofen analgesia in preclinical orofacial pain

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Abstract

Dexketoprofen (DEX) is the dextrorotatory enantiomer of S (+) configuration with a high antinociceptive activity of ketoprofen. The aim of this study was to evaluate the pharmacological interaction of DEX with the noradrenergic antagonist's prazosin, yohimbine, propranolol and atenolol in the formalin orofacial pain in mice. Analgesia to nociceptive and inflammatory pain was evaluated by dose response curves to DEX before and after the i.p. administration of 1.0 mg/kg of prazosin, or yohimbine, or propranolol or atenolol. Results are presented as means ± SEM and differences were calculated by one-way ANOVA, followed by Tukey's post-test. DEX produced a dose-related antinociceptive effect with varying potencies in both trial phases, with prazosin and yohimbine increasing the efficacy of DEX and propranolol and atenolol having no effect. These findings suggests that the increased efficacy of DEX cannot be explained by only inhibition of COXs, since it may be a consequence of multiple pharmacodynamic interactions induced by the activation of α-adrenoceptors in the opioidergic, cannabinoid, nitrergic or serotonergic mechanisms involved in pain. These results indicate that the combination of DEX with prazosin or yohimbine could be a new and effective alternative for the management of pain.

Keywords: Orofacial pain; Dexketoprofen; Prazosin; Yohimbine; Propranolol; Atenolol

1 Introduction

Pain is a sensation that habitually signals an injury or illness. The different types of pain include:

- Acute means the pain is short in duration
- Chronic is longer in duration
- Nociceptive is a type of pain caused by damage to body tissue
- Inflammatory pain is an increased sensitivity in response to mediators released by tissue damage. There are several models of experimental pain, mainly in rodents, which include acute pain tests, characterised by hot plate or tail flick and persistent or chronic pain models, represented by the formalin test. This test makes it possible to differentiate both types of pain, and can be performed in mice or rats and the administration of the formalin solution can be at the orofacial level or in the hind leg. Pain management is dominated by two classic drugs: nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids [1,2]. NSAIDs are a group of molecules with

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different chemical structures that have common pharmacological actions: analgesia, antipyresis, anti-inflammation, antiplatelet aggregation. The main mechanism of action of these drugs is the inhibition of cyclooxygenase enzymes: COX-1, COX-2 and COX-3 with the consequent decrease in the production of prostanoids: prostacyclins, thromboxanes, and prostaglandins. Among the various groups of NSAIDs, there are propionic acid derivatives formed by ibuprofen, ketoprofen, naproxen and others [3]. Propionic acid derivatives are racemic compounds, with a chiral centre because they have carbon in the α position of their structure. Ketoprofen is formed by a dextrorotatory enantiomer of S (+) configuration with a high antinociceptive activity called dexketoprofen and another R (-) enantiomer with slight analgesic activity [4].

For the preclinical study of pain, the formalin test can be used, which in its orofacial version, consists of injecting the irritating chemical agent into the upper lip of the rodent and observing the licking and scratching behavior. The formalin administration produces a biphasic painful response with a phase I (0 to 5 min) is the result of direct activation of primary nociceptive afferents, and in phase II (10 to 40 min) it is the result of inflammation-induced central sensitization in the horn dorsal spinal cord. The following mediators have been described in this test: prostaglandins (PGs), proinflammatory cytokines (ILs), the transient receptor potential ankyrin 1 (TRPA1), NMDA, and neurokinin-1 receptors, including microglial activation [5, 6].

Dexketoprofen is an NSAID that is used in various types of pain, with analgesic efficacy similar to COX-2 inhibitors and a rapid onset of action, is well-tolerated, with an opioid-sparing effect when used in multimodal analgesia pain [7,8]. The main mechanism of action is COXs inhibition. However, other associated mechanisms are not excluded, including the participation of the nitridergic and serotonergic pathways [9]. Experimental evidence of the interaction between dexketoprofen and adrenergic antagonists is scarce. Therefore, the purpose of this study was to evaluate the drug interaction of dexketoprofen (DEX) with α and β adrenoceptor antagonists using the murine model of formalin orofacial pain.

2 Material and methods

2.1 Animals

Male CF-1 mice, 35 to 40 days old, weighing 28 ± 2.0 g, housed in a light-dark cycle of 12 h at 22 ± 1 ° C, with free access to food and water, and acclimatized to the laboratory environment for at least 2 h before use. The experiments were carried out in accordance with the Ethical Guidelines of the International Association for the Study of Pain and approved by the Animal Care and Use Committee of the Faculty of Medicine (CBA 0852/FMUCH/2018). Research involving animals complied with all relevant national regulations and institutional policies for the care and use of animals. Each animal was used only once, received only one dose of the tested drugs dissolved in normal saline intraperitoneally (i.p.). All observations were made randomly and blinded. Saline control animals were interspersed at the same time as drug-treated animals, which prevented all controls from running as a single group.

2.2 Antinociception efficacy

The orofacial formalin (OF) test as described by Miranda et al., [10] was used. To perform the assay 20 µL of 2 % formalin solution were injected into the right side of the upper lip next to the nose. The chemical stimulus produces tissue injury with two distinct phases. Phase I related to the direct stimulation of nociceptors such as C fibre receptors and low-threshold mechanoreceptors including the up-regulation of substance P. Phase II related to central sensitization by an inflammatory phenomenon of the dorsal horn neurons with up-regulation of serotonin, histamine, prostaglandin and bradykinin. The nociceptive score was determined for each phase by converting the total seconds the animal spent grooming into a percentage of the maximum possible effect (% MPE), as follows:

$$\% \text{ MPE} = 100 - [(T1 - T0) \times 100]$$

where T0 and T1 are control and after treatment grooming time respectively. Control values for phase I and phase II were 95.30 ± 4.80 sec (n=18) and 120.80 ± 5.60 sec (n=18) respectively.

2.3 Experimental design

The analgesic activity of dexketoprofen (DEX) was evaluated using the OF test, from dose-response curves obtained before and after the i.p. administration of 1 mg/kg of prazosin (PRAZ) or 1 mg/kg of yohimbine (YOH) or 1 mg/kg of propranolol (PRO) or 1 mg/kg of atenolol (ATE). The drugs were administered i.p. 30 minutes prior to the test using at

least 6 animals for each of at least 4 doses. The ED_{50}, dose that induces 50% of the MPE, was calculated from a linear regression of the corresponding dose-response curve.

2.4 Drugs

Drugs were freshly dissolved in sterile physiological saline solution of 10 mL/kg, for i.p. administration. Dexketoprofen was provided by Menarini laboratories, Spain. Yohimbine hydrochloride, prazosin hydrochloride, propranolol hydrochloride and atenolol by Sigma-Aldrich Chemical Co, St. Louis, MO, USA.

2.5 Statistical analyses

Results are presented as means ± standard error of the mean (SEM). The statistical differences between the results were assessed by one-way analyses of variance (ANOVA) followed by Tukey's post-test; p values less than 0.05 ($p<0.05$) were considered statistically significant. Statistical analyses were carried out using the program Pharm Tools Pro, version 1.27, Mc Cary Group Inc., PA, USA

3 Results

The drugs used in this work did not induce significant behavioral or motor dysfunction in the mice at any of the doses used.

3.1 Antinociception induced by dexketoprofen

The i.p. administration of 1, 3, 10, 30 and 100 mg/ kg. i.p. of DEX induced dose related antinociceptive effects with different potencies in the phase I and phase II of the OF test. The dose-response curves obtained were parallel. The calculated relative potency was 2.0 times in phase I over phase II. The results are shown in figure 1.

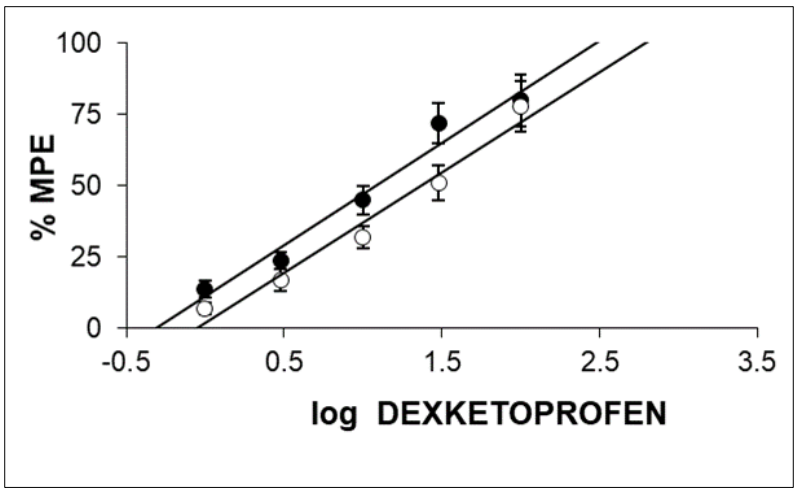

Figure 1 Dose response curves for the antinociceptive activity induced in mice by the i.p. administration of dexketoprofen, phase I (●) and phase II (○) in the formalin orofacial assay of mice. Each point is the mean ± SEM of 6-8 mice. % MPE: antinociception as percent of the maximum possible effect

3.2 Effect of noradrenergic antagonists on the antinociception of dexketoprofen

Mice pretreated with yohimbine (YOH), prazosin (PRAZ), propranolol (PRO) or atenolol (ATE) at 1.0 mg/kg i.p. did not exhibit significant changes in behavior and locomotor activity compared to controls. To determine the potency effect of noradrenergic antagonists in the efficacy of DEX in the OF assay, complete dose response curves were obtained in mice pretreated with each noradrenergic antagonist. The data revealed a significant increase in the analgesic effect of DEX by YOH and PRAZ in the OF I and II. However, no significant differences were detected with PRO and ATE in both phase of y the OF I tests (see table 1 and figure 2).

Table 1 ED_{50} values (mean ± SEM) in mg/kg and analgesic ratio (AR) for the antinociceptive activity of dexketoprofen (DEX) in mice in the formalin orofacial test (OF), before and after treatment with i.p. yohimbine (YOH) 1 mg/kg, prazosin (PRAZ) 1 mg/kg, propranolol (PRO) 1 mg/kg, atenolol (ATE) 1 mg/kg

Test	OF PHASE I	OF PHASE II	AR
Control DEX	11.9 ± 1.9	23.9 ± 3.2	2.0
Plus YOH	3.1 ± 0.8✱	13.1 ± 1.2✱	4.2
Plus PRAZ	4.2 ± 0.6✱	12.5 ± 1.7✱	2.9
Plus PRO	12.8 ± 2.8	25.5 ± 3.1	1.9
Plus ATE	12.7 ± 2.1	26.1 ± 2.8	2.0

OF I: orofacial formalin, phase I, OF II: orofacial formalin, phase II. AR: ratio between ED50 phase II / phase I. ✱ P < 0.005, compared with control

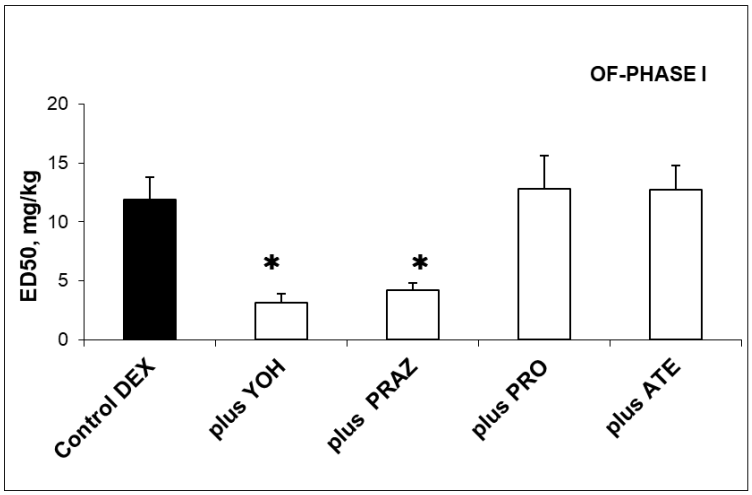

Figure 2 Effect of yohimbine (YOH), prazosin (PRAZ), propranolol (PRO) and atenolol (ATE) on the ED50 of dexketoprofen (DEX) in the orofacial formalin, phase I (OF-PHASE I), assay of mice. The ED50 obtained before and after pretreatment with YOH, PRAZ, PRO or ATE are shown in black and white columns, respectively. Columns represent the mean ± SEM of 6-8 mice. ∗: p<0.05, versus without pretreatment

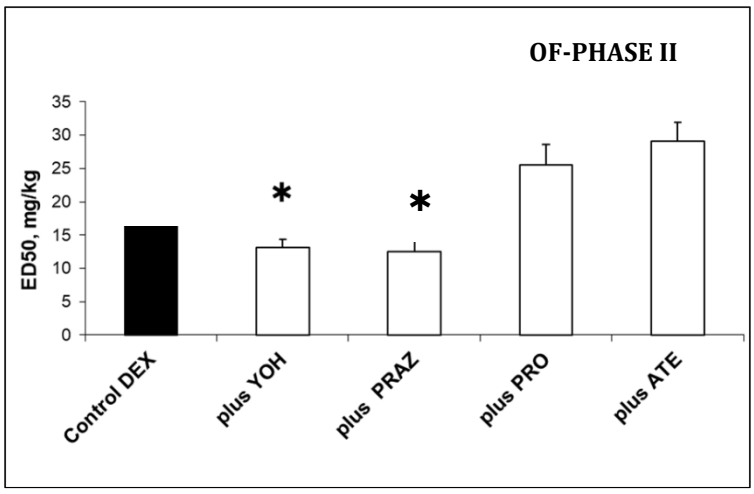

Figure 3 Effect of yohimbine (YOH), prazosin (PRAZ), propranolol (PRO) and atenolol (ATE) on the ED_{50} of dexketoprofen (DEX) in the orofacial formalin, phase II (OF-PHASE II), assay of mice. The ED_{50} obtained before and after pretreatment with YOH, PRAZ, PRO or ATE are shown in black and white columns, respectively. Columns represent the mean ± SEM of 6-8 mice. ∗: p<0.05, versus without pretreatment

In addition, the changes in the ED_{50}, expressed as the ratio between the ED_{50} values, varied between 4.2 and 1.9, as shown in table 1 and figure 3.

4 Discussion

Among the various drugs commonly used for the treatment of pain is DEX, the active dextrorotatory enantiomer of the S (+) configuration of ketoprofen. The antinociceptive efficacy of DEX has been demonstrated in different animal pain trials, regardless of the animal model or the nociceptive stimulus, either for phasic pain, such as tail flick movement or hot plate, or for tonic pain, such as the test of writhing test with acetic acid, or formalin at the level of the hind paw or orofacial. [10 -14]. The orofacial (OF) formalin-induced pain model with its two phases includes a complex multidimensional system in response to the formalin chemical stimulus that includes various types of mediators, such as transient receptor potential cation channels (TRPV1, TRPV2, and TRPM8), sensitive ions to acids acid-sensitive ion channels (ASIC), purinergic (P2X and P2Y), bradykinin (B1 and B2), N-methyl-D-aspartate (NMDA) receptors, neurokinin (NK1), calcitonin gene-related peptide (CGRP). On the other hand, it has been postulated that interleukin IL-33 participates in both phases of the assay, which by binding to its receptor induces the activation of inflammatory mediators such as IL-1b, IL-3, IL-6, TNF, IL-5, and IL-13 and multiple receptors: receptors, cannabinoids, and serotonergic, adrenergic, dopaminergic, cholinergic. [15-17]. The findings of this study demonstrate a DEX-induced dependent antinociceptive activity in both phases of the OF assay. The effect was reflected in parallel dose-response curves that could be secondary to the activation of a common mechanism of action of DEX for each phase of the algesimeter test. [18]. this finding, in agreement with Hanna and Moon [7], suggests that DEX is effective both for acute pain, through the inhibition of COX-1, represented by phase I of the OF, and for chronic pain, through of COX-2 inhibition, simulated by phase II of the same assay.

Furthermore, the results obtained from the efficacy of DEX on the biphasic response induced in the orofacial formalin assay reinforce the difference between non-inflammatory pain (phase I) and inflammatory pain (phase II).

The present study demonstrated a substantial increase in the efficacy of DEX, in both phases of the OF, reflected by a significant diminution of the ED_{50}, due to the action of PRAZ and YOH $\alpha 1$ and $\alpha 2$ adrenoceptor antagonists respectively. Nevertheless, the β1-adrenoceptor antagonists PRO and ATE lack of effect. The findings obtained with DEX can be attributed to the participation of other mechanisms in NSAID-induced antinociception in addition to prostaglandin inhibition. Like this, consequently, accordingly the participation of opioid receptors in the peripheral antinociception of dipyrone and diclofenac has been described [19]. Likewise, the participation of endogenous opioids in the analgesia of celecoxib has been shown [20]. Furthermore, the participation of the cannabinoid pathway in the analgesia induced by celecoxib has been described [20.21]. Besides, the NO / cGMP / KATP pathway has been included in the antinociceptive activity of dipyrone and diclofenac [19,22] and ibuprofen [23]. In addition, also, it has been reported that dipyrone-induced antinociception is antagonized by 5-HT2a (ketanserin), 5-HT3 (ondansetron) and 5-HT7 (SB-258719) antagonists. Besides, experimental preclinical data suggest that DEX-induced antinociception involves serotonergic mechanisms through supraspinal 5-HT (1)/5-HT (2)/5-HT (7) receptors and spinal 5-HT (3) receptors [24]. In addition, the α1-adrenoceptor antagonist prazosin, the α2-adrenoceptor antagonist yohimbine, and the β-adrenoceptor antagonist propranolol block dipyrone antinociception [25]. Moreover; yohimbine block the analgesia of ibuprofen in postsurgical pain in mice [23]. Likewise, peripheral antinociception induced by dipyrone and diclofenac was antagonized by yohimbine, prazosin and propranolol [Silva et al., 2015]. Besides, the antinociceptive activity of paracetamol in pain models is dependent on its ability to increase central cannabinoid receptors [26]. Theoretically, it is possible to suggest as an alternative to the findings presented in this work the participation of the Sigma-1 receptor (Sig-1R), which preclinical studies have shown to interact with G protein-coupled receptors and ion channels to modulate activity in the control of neuropathic and inflammatory pain [27,28].

The discrepancies in the results obtained with α- and β- adrenoceptor antagonists between the present and the cited studies could be due to the different animal species, drug doses, routes of administration, or pain models used. However, the increased effect on DEX antinociception, in addition to the inhibition of COXs, may be the consequence of a pharmacodynamic interaction induced by the activation of α-adrenoceptors in the opioidergic, cannabinoid, nitridergic, or serotonergic mechanisms already mentioned. Furthermore, the difference in results obtained with propranolol and atenolol are consistent with the proposed bidirectional modulatory effect of adrenergic receptor antagonists [29].

The described interaction of the α-adrenergic antagonists PRA and YOH with DEX suggests the activation of different and complementary mechanisms of antinociception both at the pre- and post-synaptic levels. Although the mechanisms of the antinociceptive actions of adrenergic antagonists are complex, the results of this work support the conclusion that DEX induces an antinociceptive activity that can be modulated by the α-noradrenergic receptor systems.

5 Conclusion

This study demonstrates that there is a functional interaction between α-noradrenergic antagonists and the antinociceptive properties of DEX in murine orofacial formalin tonic pain. This interaction proposes that the α-noradrenergic system modulates the analgesic efficacy of DEX in the acute and chronic action and appears to be mediated by the multiple mechanisms of action. These results allow us to suggest that the combination of DEX with α-noradrenergic antagonists may represent a new and effective alternative for the therapeutic management of pain.

Abbreviations

DEX: dexketoprofen, NSAIDs: non-steroidal anti-inflammatory drugs, COX: cyclooxygenase, PGs: prostaglandins, ILs: interleukins, TRPA1: transient receptor potential ankyrin 1, NMDA: N-methyl-D-aspartate, OF: orofacial formalin, PRAZ: prazosin, YOH: yohimbine, PRO: propranolol, ATE: atenolol, ED_{50}: effective dose 50, NK1: neurokinin, CGRP: calcitonin gene related peptide, TRPV: transient receptor potential cation channels, ASIC: sensitive ions to acids acid-sensitive ion channels,P2X, PSY: purinergic receptors, B1, B2: bradykinin receptors, NO / cGMP / KATP: nitric oxide (NO)-cyclic guanosine monophosphate (cGMP)/ ATP-sensitive potassium *channel, 5-HT: serotonin, Sig-1R: sigma-receptor.*

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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Authours´ contributions

All authors contributed directly and substantially to the study and approved the final version of the manuscript.

Statement of ethical approval

Hereby, Hugo F. Miranda, I assure that the manuscript: "NITRIDERGIC MODULATION OF COX-2 EFFICACY" complies with being an original research work, that has not been sent for publication elsewhere, that it has been prepared by all the authors and are responsible for their content. All animal procedures were performed in accordance with the ethical guidelines of the International Association for the study of pain and approved by the animal care and use committee of the Faculty of Medicine, University of Chile, (CBA 0852/FMUCH/2018).

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