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Nitric Oxide Mediated Dexketoprofen Antinociception

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ABSTRACT

Nonsteroidal anti-inflammatory drugs (NSAIDs) are often used in the treatment of pain by their analgesic, anti-inflammatory and antipyretic properties. NSAIDs act via inhibition of cyclooxygenase enzymes, COX-1, COX-2 and COX-3. In this study, the antinociceptive activity of the dextrorotatory enantiomers of S (+) configuration of ketoprofen, denominate, dexketoprofen (DEX), was evaluated, before and after the pretreatment of mice with N(ω)-nitro-L-arginine methyl ester (L-NAME), in two models of pain. One model of tonic pain, the tail flick (TF) assay and in a second model of phasic pain, the acetic acid writhing test (WT). (DEX) administration produced a dose-dependent antinociceptive effect in both murine assays, but with different potency. The dose that produced 50 % of maximum possible effect (ED_{50}) of antinociception of the WT was 3.87-fold more potent than TF. The pretreatment of mice with 1, 3 or 10 mg/kg, i.p of L-NAME produced a significant decrease of the antinociceptive effect of DEX, reflected with an important increase of ED_{50} in both assays. In conclusion, the results the application of DEX produced antinociception in the WT and TF models and in this effect the activation of NO pathway plays an important role.

KEYWORDS: Nociception, dexketoprofen, L- NAME, writhing test, tail flick assay.

INTRODUCTION

Experimental pain models include animal tests for acute pain and persistent pain. For acute pain, tests such as the hot plate or tail flick are used, while for persistent pain the formalin test or the acetic acid contortion test can be used. These tests perceive the harmful effects of the stimulus through the peripheral nervous system. Nociception and pain are major fields of both neuroscience and medical research, as several rodent trials have been used to generate tools for research. Hence, thermal, mechanical, and chemical stimuli, as well as measurements of hyperalgesia and allodynia, models of inflammatory or neuropathic pain, are part of the toolbox available to researchers (Le Bars *et al.* 2001; Barrot, 2012).

Among the methods used to induce noxious effect in rodents are the tail movement test (TF) and the acetic acid test (WT). TF is one of the oldest nociceptive tests of tonic pain due to the long duration of the stimulus and is a spinal reflex. The WT, also an old test for phasic pain due to the short duration of the stimuli, is a chemical test for visceral pain. The antinociceptive activity is induced by the intraperitoneal administration of an acetic acid solution that causes a typical behavior in the rodent (Barrot 2012, Turner et al. 2019).

The most widely used medications for pain therapy are NSAIDs and opioids. In NSAIDs, due to their chemical structure, there are several groups, among them, derivatives of propionic acid (ibuprofen, naproxen, ketoprofen). The members of propionic acid, have a chiral center, due to an asymmetric carbon, converting NSAIDs into racemic compound. NSAIDs work primarily by suppressing the enzymes cyclooxygenase: COX -1, COX-2 and COX-3, nevertheless, the antinociceptive effects of NSAIDs have been shown to be based not only on COX inhibition but also on other molecular mechanisms that cooperate to explain NSAID-induced analgesia (Dwivedi et al. 2015; Gunaydin and Sim Bilge, 2018).

The NSAIDs used in the pharmacotherapy of pain meet certain aspects of their selection such as potency, selectivity for the COX isoform, pharmacodynamic interactions, safety, side effects, and more. For

this reason, racemic NSAIDs, such as ketoprofen, provide broad antinociceptive results, since dextrorotatory enantiomers of S (+) configuration, called dexketoprofen, have a high antinociceptive activity, since the other R (-) enantiomer retains a low antinociceptive power (Hardikar, 2008). There are several evidences that somatic and visceral pain is associated with nitric oxide (Abacioğlu et al. 2000; Cury et al. 2011; Staunton et al. 2018; Spiller et al. 2019; Noriega et al. 2020).

The objective of the current study was to evaluate the involvement of nitric oxide (NO) a controversial molecule with dual effects, pronociceptive and antinociceptive in the antinociception induced by dexketoprofen in mice by the radiant heat tail flick and the chemical acetic acid induced writhing test of mice.

MATERIALS AND METHODS

Animals

Male CF-1 mice weighing 25-30 g were tested divided randomly into groups of 6-8 mice. Animals were housed on a 12:12 h light-dark cycle at 22 ± 1° C with access to food and water ad libitum. Mice were acclimatized to the laboratory environment for at least 1 h, each animal was used in one experiment only and euthanized by overdose of anesthetic (pentobarbital intraperitoneally (i.p.) 60 mg/kg) immediately following the algesimeter test. All protocols were approved by the Animal Care and Use Committee at the Faculty of Medicine, University of Chile (Protocol CBA 0852/FMUCH/2018). All experiments were performed by research blind to drug treatment.

Antinociception

The nociceptive tests used were the tail flick (TF) and the acetic acid writhing test (WT). The TF test was assessed as previously described (Miranda et al., 2007) using an digital algesimeter (U. Basile, Comerio, Italy). The animal withdraws its tail in response to the heat applied, this reaction time is the tail flick latency. The prolongation of the reaction time is established as antinociceptive activity and a cut-off time of 8 seconds was established to avoid damage to the tail of the animal. The tail flick latency was recorded prior to drug administration (control latency or baseline value: 3.10 ± 0.17 sec, n= 18) and at 30 minutes after i.p. drug administration. The antinociceptive response was calculated as percent of maximum possible effect (% MPE), where

$$\%\,\mathrm{MPE} = [(\mathrm{test} - \mathrm{control}) / (8 - \mathrm{control})] \times 100.$$

In the assay of the WT the procedure used has been described previously (Miranda et al., 2007), in which mice were injected i.p. with 10 mL/kg of 0.6 acetic acid solution. The chemical stimulus induces a wave of contraction of the abdominal muscles followed by the extension of the hind limbs (writhes) and a reduction in motor activity and motor incoordination and the number of writhes in a 5 min after the i.p. chemical solution was counted. Antinociception is expressed as percentage of inhibition of the number of writhes obtained in control animals (23.92 ± 1.41, n = 18).

Protocol

Dose response curves, i.p. for dexketoprofen (3, 10, 30, 100, 300 mg/kg) for TF and (1, 3,10,30 and 100 mg/kg) for WT were obtained before and after pretreatment of mice with 1, 3 or 10 mg/kg i.p. of L-NAME. A squares linear regression analysis of the log dose response curve allowed the calculation of the doses that produced 50 % of antinociception of dexketoprofen (Ed_{50}).

Drugs

Drugs were freshly dissolved in 10 mL/kg of sterile physiological saline solution. Dexketoprofen trometamol (DEX) was provided by Menarini Laboratory, Spain; N-nitro-L-arginine methyl ester (L-NAME) was purchased from Sigma-Aldrich Chemical Co, St. Louis, MO, USA.

Statistical analysis

Results are presented as means ± SEM. The statistical difference between before and after the pretreatment with L-NAME was assessed was by Student's test for independent means, *p* values less than 0.05 ($p < 0.05$) were considered statistically significant. Results analyzed used Pharm Tools Pro, version 1.27,

McCary Group Inc, PA, USA.

RESULTS

Antinociception by dexketoprofen

The i.p. administration of DEX produced a dose-dependent antinociceptive effect in both murine assays, with different potency. Thus, in the TF test, the increase in the control latency time was accompanied with an ED_{50} of 48.06 ± 1.73 mg/kg. In addition, in the WT, the AINE induced a significant decrease of the writhes complemented $_{with}$ an ED_{50} of 12.41 ± 0.32 mg/kg. The ED_{50} of WT was 3.87-fold more potent than TF (all results in **Table** 1).

TABLE 1: ED_{50} values (means ± SEM) and potency ratio for the antinociceptives activity of i.p. DEX after pretreatment with 1, 2 and 3 mg/kg, i.p. of L-NAME in the TF and WT assays.

Drug	TF		WT	
	ED_{50} (mg/kg)	Ratio	ED_{50} (mg/kg)	Ratio
Dexketoprofen	48.06 ± 1.73	1	12.41 ± 0.32	1
Plus, L-NAME 1 mg/kg	63.05 ± 2.09	1.37	20.66 ± 0.97	1.66
Plus, L-NAME 3 mg/kg	84.74 ± 7.31	1.70	25.60 ± 3.76	2.06
Plus, L-NAME 10 mg/kg	105.02 ± 9.66	2.19	33.10 ± 4.30	2.67

Ratio was compared with dexketoprofen control.
WT: acetic acid writhing test. TF: tail flick assay.

Effect of L-NAME

The pretreatment of mice with 1, 3 or 10 mg/kg, i.p of L-NAME resulted in a significant decrease of the antinociceptive effect of DEX, reflected in an important increase of ED_{50} in the TF and the WT assays, as can be seen in **Figure 1.**

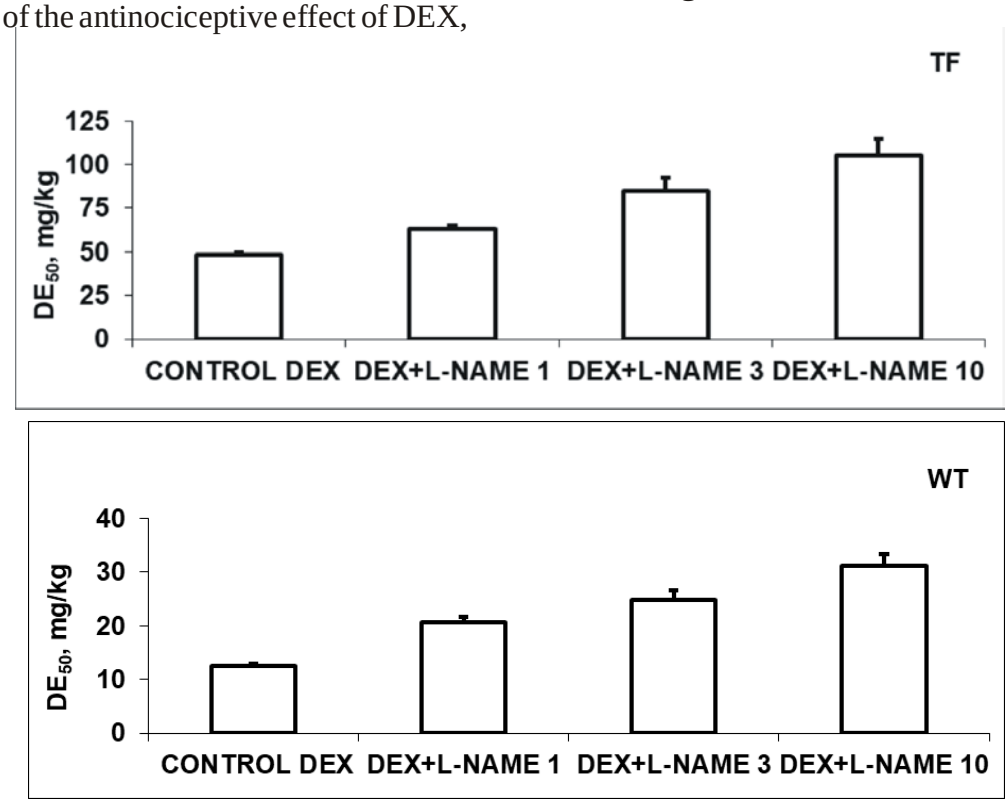

Figure 1. Effect of intraperitoneal administration of 1, 3 and 10 mg/ kg of L-NAME on the ED_{50} of antinociception of DEX in the WT and TF assay of mice.

The dose-dependent curves for each pretreatment wit L-NAME are displayed in **Figures 2 and 3.** However, no significant difference was found in the values of the relative potency of DEX after treatment of mice with L-NAME, calculated based on the DE_{50} displayed in TF and WT, see **Table 1**.

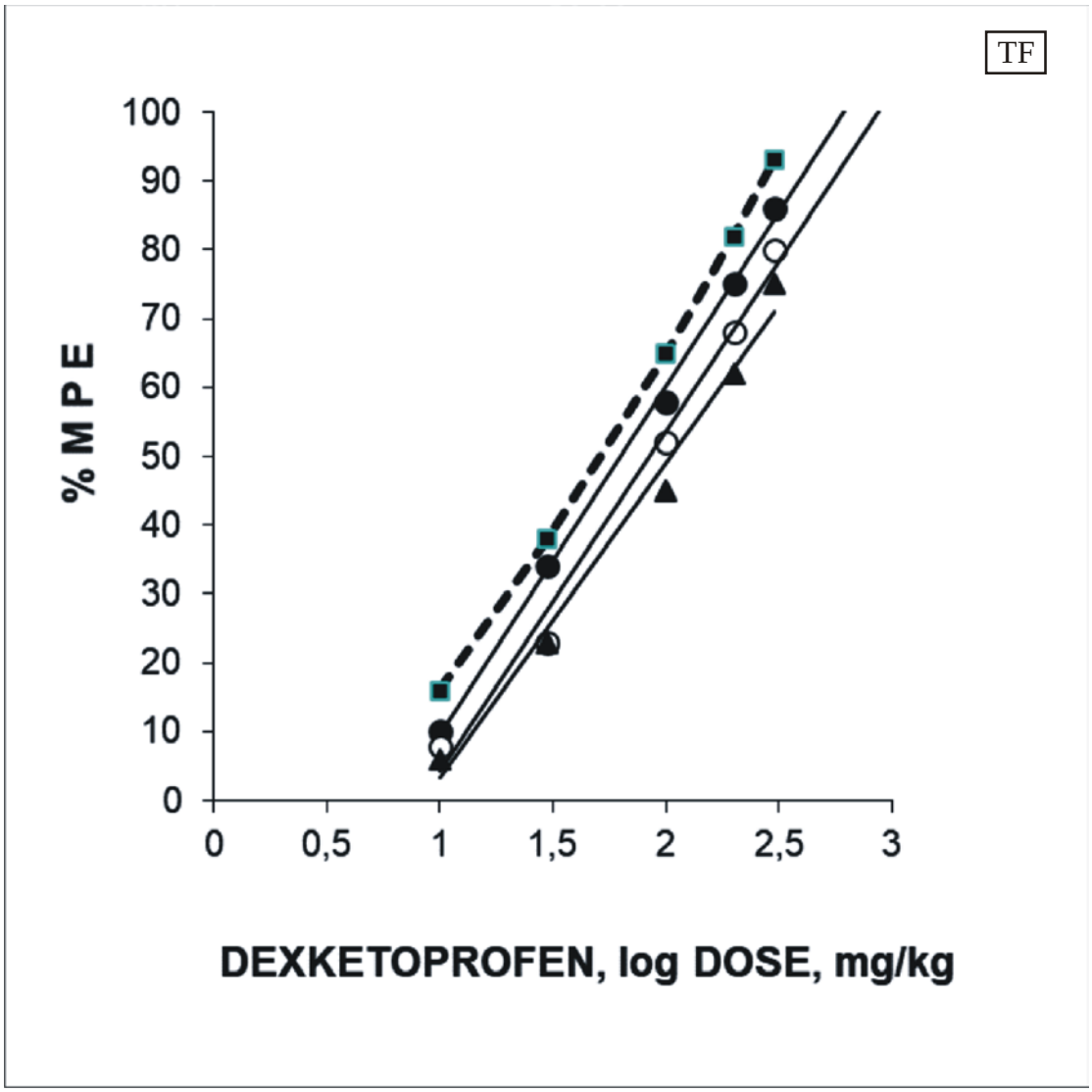

Figure 2. Dose-response curves of DEX antinociceptive activity before and after pretreatment with 1, 3 and 10 mg / kg of L-NAME. (■) DEX control, ( ) DEX + L-NAME 1, ( ) DEX + L-NAME 3, ( ) DEX + L-NAME 10 in the TF test of mice. Each point is the average of 6-8 mice. % MPE: antinociception as a percentage of the maximum possible effect.

Each point is the mean of 6-8 mice. % MPE: antinociception as percentage of the maximum possible effect.

DISCUSSION

NSAIDs are a group of drugs that have analgesic, anti-inflammatory and antipyretic properties and are widely used for the treatment of pain, but are limited by their ceiling effect, so an alternative is combined use, in the so-called multimodal analgesia. The results of these studies, carried out in both humans and animals, have been contradictory, as some have reported synergism (Miranda et al. 2004; Oh et al. 2016; Zapata-Morales et al. 2016; Fornasari et al. 2017; Ortiz, 2017; Merlos et al. 2018; Boakye-Gyasi et al. 2018; Lin et al. 2019) and others reported lack of interaction (Rhu et al.

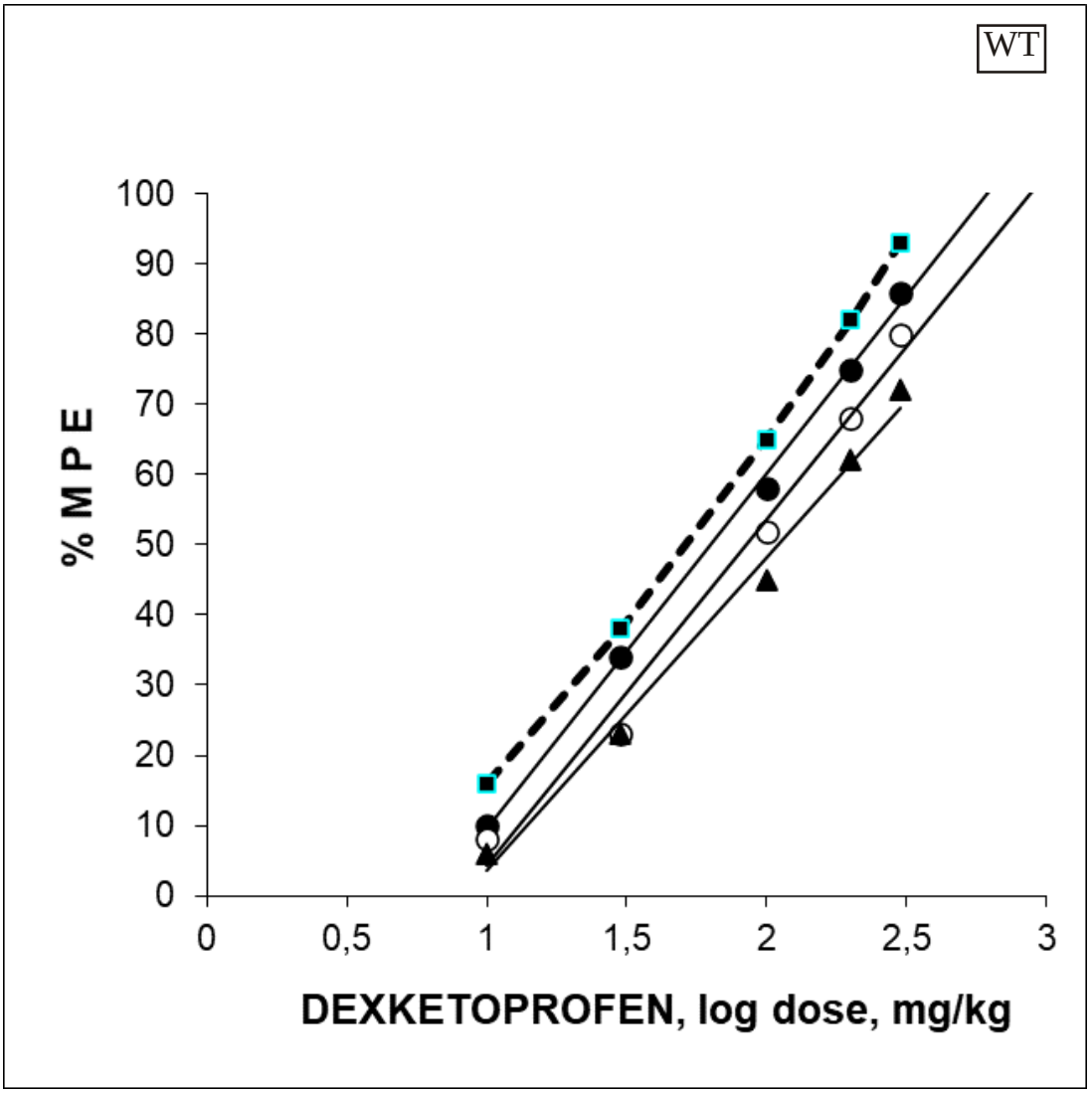

Figure 3. Dose-response curves of DEX antinociceptive activity before and after pretreatment with 1, 3 and 10 mg / kg of L-NAME. (■) DEX control, ( ) DEX + L-NAME 1, ( ) DEX + L-NAME 3, ( ) DEX + L-NAME 10 in the mouse WF test. Each point is the average of 6-8 mice. % MPE: antinociception as a percentage of the maximum possible effect.

Furthermore, the antinociceptive activity of NSAIDs seems not to be exclusively due to COX inhibition, since other mechanisms have been reported that would co-help with this effect. These may include affinity for phospholipase A2 (PLA2), prostaglandin keto reductase (PTGR), lactoperoxidase (LPO), transthyretin (TTR), lactoferrin (LF), interactions with nitric oxide, monoaminergic, serotonergic pathways, down-regulation of L-selectin, inhibition of NF-kappa-β, modulation of IL-β, IL-6 and others (Hamza and Dionne, 2009; Diaz-Gonzalez and Sanchez-Madrid, 2015; Dwivedi et al. 2015; Gunaydin and Sim Bilge, 2018).

The aim of the current study was to evaluate the antinociception induced by DEX and the interaction with L-NAME. Results advises that, as in previous studies, it was obtained a dose-dependent antinociceptive activity of DEX in TF and WT (Abacioğlu et al. 2000; Miranda et al. 2007; Miranda et al. 2008; Lu et al. 2014; Noriega et al. 2020). The differences in DEX potency may be due to the type of pain model evaluated: one phasic and the other tonic. Pretreatment of mice with different doses of L-NAME induced a dose-dependent decrease in DEX antinociceptive potency reflected by a sequential increase in ED50 value, up to 3.17-fold and 2.67-fold in the TF and WT assays, respectively. However, L-NAME did not induce changes in the slope of

DEX antinociceptive dose-response curves. The effect of L-NAME is related to the NO activity, since there is evidence that somatic and visceral pain is associated with NO. Moreover, it has been reported that NO is a key modulator of pain and performs nociceptive and antinociceptive functions (Abacioğlu et al. 2000; Sousa and Prado, 2001; Espluges, 2009; Cury et al. 2011; Spiller et al. 2019). NO produces its effect at the nocifensive level appears to be dependent on the isoforms of the enzyme nitric oxide synthase (NOS): two are constitutive neuronal NOS (nNOS) and endothelial NOS (eNOS) while the third is inducible (iNOS) (Cinelli et al., 2020). Consequently, it has been reported that the pharmacological control of pain is dependent on the inhibitory action of COXs on prostaglandins and the blocking by action of NO of the sensitization of nociceptors (Gomes et al. 2020) and also that the effects of several analgesic drugs were antagonized by the neural elective inhibitor n-NOS but not by the other NOS isoforms (Thiago et al. 2011). On the other hand, according to the results, they suggest that L-NAME could exert its analgesic effects by reducing NOS and altering the balance between proinflammatory (IL-1β and IL-1α) and anti-inflammatory (IL-10) cytokines (Staunton et al. 2018).

The results demonstrated that i.p. DEX had a significant antinociceptive effect in the tail flick and acetic acid-induced writhing tests indicating the involvement of peripheral and central analgesic mechanisms. The significant increase of ED_{50} after pretreatment with L-NAME suggests a NO involvement in DEX antinociception.

CONCLUSION

In conclusion, the i.p. administration of DEX produced antinociception in the WT and TF models and in this effect seems modulate by the activation of NO pathway since i.p. pretreatment of mice with L-NAME plays an important role in the antinociception activity induced by dexketoprofen.

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