OPIOID MODULATION OF ANTINOCICEPTION OF CELECOXIB AND KETOROLAC AT PRE-CLINICAL PAIN

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ABSTRACT

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used to treat pain, fever, and inflammation. Among them are celecoxib and ketorolac, whose efficacy is dependent on the inhibition of COXs and seems to be modulated by additional mechanisms that would contribute to analgesic efficacy. On the other hand, the interaction of NSAIDs with other types of analgesics, especially with opioids, is often poorly identified. The objective of this work was to evaluate the effect of the opioid antagonists: naltrexone, naltrindole and nor-binaltorphimine on the efficacy of celecoxib and ketorolac in murine models of tonic and visceral pain induced by chemical stimuli, such as contortions induced by acetic acid and the formalin hind paw test. The antinociceptive potency of celecoxib and ketorolac was assessed using a dose-response curve from 0.1-10 mg/kg, i.p. before and after pretreatment of mice with 1 mg/kg i.p. of the opioid antagonist's naltrexone, naltrindole, or norbinaltorphimine. Celecoxib was 1.58 to 4.85 times more potent than ketorolac. The effect of both NSAIDs was unequally modified by opioid antagonists. Thus, the efficacy of ketorolac increased in the writhing test and in the phase I formalin trial, while that of celecoxib only increased in the phase II formalin trial. The results demonstrate that ketorolac and celecoxib induce significant antinociception, whose efficacy was increased by specific opioid receptor antagonists, suggesting that the antinociception induced by ketorolac and celecoxib is dependent on opioid receptors.

KEYWORDS: Celecoxib, ketorolac, antinociception, opioid's antagonist, chemical pain.

INTRODUCTION

The main action of non-steroidal anti-inflammatory drugs (NSAIDs) is to treat fever, inflammation, and pain. These drugs inhibit cyclooxygenase enzymes (COXs), which are the fundamental centre for the synthesis of prostanoids: prostaglandins, prostacyclin and thromboxane. Three isoforms of COX have been described; cyclooxygenase-1 (COX-1) considered constitutive; it is expressed in most tissues. Protects the gastrointestinal tract and induces platelet aggregation in response to injury. COX-2 nominated as inducible, as it is mostly undetectable in tissues; it is significantly increased during the inflammatory process. The COX-3 isoform described as a variant of COX-1 expressed in nerve tissues, endothelial cells and heart. Most NSAIDs are non-selective COX-1 inhibitor (Chandrasekharan et al.2002; Gunaydin and Bilge, 2018).

Ketorolac with strong analgesic, antipyretic, and anti-inflammatory properties has been used clinically to treat post-operative, inflammatory, and neuropathic pain It is used for the short- term treatment of postoperative pain, arthritis, menstrual disorders, and headaches, among other isorders. This NSAID is related to a reduction in behavioural sensitivity, nevertheless does not induce a risk of dependence or tolerance (Macario and Lipmanm 2001; Vacha et al. 2015). Celecoxib, a selective inhibitor for the COX-2isoform with analgesic, antipyretic, and anti-inflammatory effects similar to those of non-selective pain caused by injuries, surgeries, and other medical or dental procedures. The systematic review provides evidence on the benefits and safety of celecoxib compared to placebo or NSAIDs in various types of pain, such as rheumatoid arthritis, ankylosing spondylitis and others (Fidahic et al. 2017).

The marked antinociceptive activity of celecoxib and ketorolac, in addition to being dependent on COX, has led to the suggestion that the analgesic activity could be facilitated by additional mechanisms that would contribute to the modulation of antinociception. Among these, possibly through the opioid system. Due to the scarcity of reports of experimental pharmacological interaction between NSAIDs and opioids, in the present study, it was evaluated, in two murine models of pain induced by chemical stimulation, the modulation of naltrexone, naltrindole, and nor-binaltorphimine in antinociception induced by celecoxib and ketorolac.

MATERIALS AND METHODS

Experimental animals

Male CF-1 mice (25-30g) were used with free access to food and water ad libitum and housed on a 12 h light-dark cycle at 22±1 °C. All procedures with animals from the central nursery were approved by the Animal Care and Use Committee of the Faculty of Medicine, University of Chile (Protocol 852/FMUCH/2018). Animals were tested divided randomly into group of 6-8 mice and acclimatized to the laboratory for at least 1 h before testing, used only once during the protocol, and euthanized after the algesimeter test by one intraperitoneal (i.p.) injection of 60 mg/kg of pentobarbital. The number of animals was kept at a minimum, compatible whit consistent effects of the drug treatment.

Measured of antinociception

Antinociception was assessed by the following murine tests: (A) acetic acid writhing test (WT) as described previously (Miranda et al. 2006). In this test, the mice were injected i.p. with 10 ml of 0.6 ml acetic acid. The chemical stimulus induces a wave of

contraction of the abdominal muscle followed by extension of the hindlimbs (writhes) and reduction in motor activity. The number of writhes in 5 min after the i.p. of chemical solution was counted. Antinociception is the percentage of inhibition of the number of writhes in control mice (22.8 ±1.4, n=24) and converted to % MPE (percentage of maximum possible effect). (B) the formalin hind paw (FHP) test described previously was used (Miranda et al. 2007). To perform the test 20 µL of 2 % formalin solution was injected into the dorsal surface of the right hind paw. The intensity of pain was assessed as the time, in sec, by the licking or biting of the injected paw. The test show 2 periods: phase I, corresponding to the 5 min immediately after formalin injection and phase II, chronicled by 10 min, a period starting 20 min after formalin injection. The control values were, phase I: 123.05 ± 8.40 sec, (n =12) and phase II: 157.63 ± 9.10 sec, (n=12). Licking time was converted to % MPE.

Experimental protocol

The antinociceptive potency of celecoxib and ketorolac was assessed by means a dose-response curve from 0.1, 0.3, 1, 3 and 10 mg/kg, i.p. the WT and FHP tests using at least 6 animals for each at least 4 doses, as can be seen in figure 1. The DE_{50}, dose that induce 50% of MPE, was calculated from lineal regression of dose-response curves of celecoxib and ketorolac before and after the pretreatment of mice with 1 mg/kg i.p. of each of the naltrexone (NTX), naltrindole (NTI) or nor-binaltorphimine (nor-BNI).

Drugs

Drugs were freshly dissolved in sterile physiological salt solution of 10 mL/Kg, for i.p. administration. Celecoxib and ketorolac tromethamine from Laboratory Chile.

Naltrexone hydrochloride, naltrindole hydrochloride and nor-binaltorphimine dihydrochloride were obtained from Sigma-Aldrich Chemical Co, St. Louis, Mo, USA.

Statistical analysis

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

RESULTS

Antinociception induced by celecoxib and ketorolac

The administration i.p. of celecoxib and ketorolac produced a dose related antinociceptive activity with different potencies in the WT and in phase I and II of the FHP assays of mice (see Fig.1). Celecoxib resulted to be 1.58 times more potent than ketorolac in WT, 1.35 times in FHP phase I and 4.85 times in FHP phase II. This relative potency, expressed as ED_{50}, can be seen in Table 1.

Opioid antagonists' effect in the efficacy of celecoxib and ketorolac

Mice treated with 1 mg / kg i.p. of NTX, or NTI or nor-BNI did not modify the behavior of the control mice. To determine the interaction of opioid antagonists a curve dose-response to celecoxib and ketorolac, was performed in WT and FHP tests. Pretreatment of mice with 1 mg/kg i.p. of NTX, an enhanced analgesic efficacy was obtained by ketorolac in the WT and FHP-I through of a significant shift of the ED_{50}. A similar effect was obtained with NTI (1 mg/kg i.p.) and nor-BNI (1 mg/ i.p.). While pretreatment of mice with NTX,

NTI and nor-BNI did not significant change the ketorolac ED_{50} value in FHP phase II. All these results can be seen in Tables 1 and 2.

In the case of celecoxib, only pretreatment of mice with 1 mg/kg i.p. of nor-BNI enhanced analgesic efficacy was obtained in the FHP phase II assay by a significant modification of ED_{50}. However, pretreatment of mice with 1 mg/kg i.p. of NTX or NTI did not induced variation of the ED_{50} value of celecoxib in WT or phase I of FHP. All these results can be seen in Table 1 and 2.

DISCUSSION

NSAIDs are widely used drugs in pain therapy, generally administered alone, since their combinations are not used frequently due to their contradictory results. As an example, it has been reported that the interaction of NSAIDs with opioids results between non-interacting, additive, or synergistic.

Therefore, in the present study to evaluate the interaction between two NSAIDs and their possible mechanism of action, it was confirmed the antinociceptive and anti-inflammatory properties of both ketorolac and celecoxib, respectively. These findings are consistent with previous reports of experimental pain such as tail flick, hot plate, formalin, carrageenan model, and neuropathic tests, among others (Domer, 1990; Tejwani and Rattan, 2000; Nishiyama, 2006; Kausar and Davis, 2006, Correa et al. 2010; Zhao et al. 2017; Vicente-Baz et al. 2019). However, it is necessary to emphasize that the lack of effect of celecoxib was not detected in the first phase of the formalin hind paw test reported by Zhao et al. 2017.

The current study shows that the efficacy of ketorolac was increased by pretreatment of mice with NTX and NTI in WT and FHP-I, which could be explained by a possible

opioid modulation developed by MOR and DOR opioid receptors in the antinociceptive activity of this NSAID. However, the opioid receptor KOR, through nor-BNI, lacks this modulatory effect on ketorolac antinociception in the FHP-II assay. On the other hand, it is found that the MOR and DOR opioid receptors lack a modulating effect on the analgesic activity of celecoxib and that its efficacy is only increased by the action of KOR in the FHP-II trial.

The differences in responses can be explained by the type of stimuli and the pain generated by the models used. Thus, the test of abdominal contortions induced by the administration of an acetic acid solution is a noxa that directly stimulates peripheral nociceptors followed by localized visceral inflammation due to the release of mediators via prostaglandins. On the other hand, the two phases of FHP are associated with two different mechanisms of nociception: the first phase is by direct stimulation of nociceptors through the direct activation of transient ankyrin receptors (TRPA)-1, such as C fibre and low-threshold mechanoreceptors, including the upregulation of substance P. Meanwhile, the second phase reflects the combination between peripheral and central actions, including neuronal and glial responses, including the upregulation of serotonin, histamine, prostaglandin, and bradykinin (Tejwani and Rattan, 2000; Nishiyama, 2006; Kausar and Davis, 2006, Correa et al. 2010; Zhao et al. 2017; Vicente-Baz et al. 2019; Yam et al., 2020). x These differences in the type of pain induced in each assay could be modified by the intrinsic inhibitory properties of COX isoenzymes of each NSAIDs.

Other alternatives to explain the differences in antinociceptive activity reported between the two NSAIDs could be related to:

(A) the inhibitory effect that ketorolac exerts on NO in the activation of NMDA, a synaptic pain modulator (Kausar and Davis, 2006).

(B) the fact that it has been suggested that ketorolac might causes release of endogenous opioids, such as met-enkephalin. However, ketorolac was not able to inhibit the binding of specific ligands to MOR, DOR and KOR opioid receptors (Tejwani and Rattan, 2000; Nishiyama, 2006; Kausar and Davis, 2006, Correa et al. 2010; Zhao et al. 2017; Vicente-Baz et al. 2019; Yam et al., 2020; Pirkulashvili et al. 2017; Gorgiladze et al. 2017; Tsagareli et al. 2020). Though, the present findings are not in agreement with the report that naloxone, antagonist with a high affinity for the MOR-opioid receptor, prevents the analgesic effect of ketorolac in the formalin assay. The reason for this difference could be due to differences in the experimental protocols or to changes in other structures or in mediators such as endogenous opioid peptides (Pirkulashvili et al. 2017; Gorgiladze et al. 2017; Tsagareli et al. 2020).

(C) the reducing activity of ketorolac on astrocyte activation and associated expression of the inflammatory mediator PAR1 (Dong et al. 2013).

(D) the analgesic activity of celecoxib is mediated by peripheral mu, kappa, and delta opioid receptors (Correa et al. 2010).

(E) In addition to its COX-inhibition activity, celecoxib has been reported to interact with ionic channels such as Na^+ currents and Kv7 channels (Park et al. 2007; Du et al. 2011; Frolov and Sing, 2014: Vicente-Baz et al. 2019).

(F) Other mediators that, in addition to the inhibition of COXs, that contribute in the antinociception activity of NSAIDs, among them: i-NOS, IL-1 β, IL-6, TNF-□ and others (Hamza and Dionne, 2009; Gunaydin and Bilge, 2018).

CONCLUSION

In conclusion, the results of this study indicate that the administration i.p. of ketorolac and celecoxib induces both antinociception and anti-inflammation. Efficacy is increased by pretreatment with the specific opioid receptor antagonist's naltrexone, naltrindole, and nor-binaltorphimine. The results could be explained by the different pharmacological mechanisms attributed to NSAIDs. The findings demonstrate that the opioid receptors MOR, KOR, and DOR may play a role in the antinociceptive and anti-inflammatory activity induced by ketorolac and celecoxib.

All authors contributed direct and substantially to the study. Approved the final version of the manuscript. The authors have no conflicts of interest to declare.

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LEGEND TO FIGURE

Figure 1. Dose-response for the antinociceptive activity induced by ketorolac (●) and by celecoxib (O) in the acetic acid writhing test (WT) and formalin hind paw (FHP), phase I and II assays of mice. Each point is the mean of 6-8 mice. % MPE represent antinociception evaluated as percentage of maximum possible effect. Abscissa is log of dose of ketorolac or celecoxib