European Journal of Pharmacology 805 (2017) 51–57

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

Low doses of tizanidine synergize the anti-nociceptive and anti-inflammatory effects of ketorolac or naproxen while reducing of side effects

Selene I. Patiño-Camacho[a,b], Myrna Déciga Campos[a,*], Karla Beltrán-Villalobos[a], Dalia A. Castro-Vidal[c,d], Rosa M. Montiel-Ruiz[e], Francisco J. Flores-Murrieta[a,f]

[a] Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Instituto Politécnico Nacional, Ciudad de México, Mexico
[b] Centro de Investigación para el Desarrollo Industrial, Universidad Autónoma de Guadalajara, Jalisco, Mexico
[c] Facultad de Química, Universidad Autónoma de Yucatán. Yucatán, Mexico
[d] Facultad de Ciencias Químicas e Ingeniería, Universidad Autónoma de Baja California, Baja California, Mexico
[e] Centro de Investigación Biomédica del Sur, IMSS, Argentina No.1 Col. Centro Xochitepec, Morelos, Mexico
[f] Unidad de Investigación en Farmacología Clínica, Instituto Nacional de Enfermedades Respiratorias, S.S.A., Ciudad de México, Mexico

ARTICLE INFO

Keywords:
Tizanidine
Naproxen
Ketorolac
Combination
Synergism
Anti-inflammatory effect
Anti-nociception

ABSTRACT

The aim of the present study was to determine whether tizanidine, an alpha2-adrenoceptor agonist, is able to increase the anti-inflammatory and anti-nociceptive effects of naproxen and ketorolac with a low incidence of gastric injury and spontaneous activity in rats. The anti-inflammatory effect was assayed in a carrageenan test, and oral administration of tizanidine (ED_{40} =0.94 ± 0.2 mg/kg), naproxen (ED_{40}=3.18 ± 0.4 mg/kg), and ketorolac (ED_{40}=16.4 ± 1.9 mg/kg) showed a dose-dependent effect on inflammation. The anti-nociceptive effect was assayed in the formalin test, and administration of tizanidine (ED_{40}=0.39 ± 0.06 mg/kg, p.o.), naproxen (ED_{40}=33.9 ± 3.9 mg/kg, p.o.) or ketorolac (ED_{40}=6.49 ± 1 mg/kg, p.o.) each showed a dose-dependent anti-nociceptive effect. The effects of combinations of tizanidine/naproxen and tizanidine/ketorolac were determined considering their ED_{40} at a rate of 1:1. Additionally, the tizanidine/naproxen and tizanidine/ketorolac combinations showed anti-inflammatory and anti-nociceptive effects. The tizanidine/ketorolac combination was more potent than tizanidine/naproxen, in both inflammatory (interaction index=0.03 tizanidine/ketorolac and 0.07 tizanidine/naproxen) and nociceptive (interaction index=0.005 tizanidine/ketorolac and 0.01 tizanidine/naproxen) processes. In both cases, tizanidine improved naproxen and ketorolac gastrointestinal tolerability by 50%. Furthermore, co-administration of tizanidine with naproxen or ketorolac did not modify the spontaneous activity in the same way as individual tizanidine administration. Considering that tizanidine increases the anti-inflammatory and anti-nociceptive effects of naproxen or ketorolac, with an increase in gastric tolerability, tizanidine could provide therapeutic advantages in the clinical treatment of inflammation and pain.

1. Introduction

Naproxen and ketorolac belong in the nonsteroidal anti-inflammatory drug group with higher anti-nociceptive and anti-inflammatory properties. Current literature contains much data about both drugs being used in chronic pathologies related to the inflammatory process, primarily pain and migraines (Freitag, 2003; Carvalho et al., 2013; Ekman et al., 2014; Essex et al., 2015, 2012; Buccelletti et al., 2014; Bugada et al., 2015). Ketorolac is used in the treatment of persistent postsurgical pain and of non-traumatic and post-traumatic pain. There are also many reports concerned with the adverse effects of ketorolac and naproxen in chronic pathologies; patients present adverse effects that are costly and decrease their quality of life. The principal adverse effects for these drugs are gastric and renal damage, hepatotoxicity, and thrombotic cardiovascular events, and insolated case reports have documented seizures, hypotension and apnea (Chan et al., 2006; Robich et al., 2011).

This paper presents an alternative therapy for treating inflammatory pain: the use of low doses of naproxen and ketorolac combined with an additional drug. Tizanidine is a centrally acting myospasmolytic drug that activates the central alpha2-adrenoceptor (Nabeshima et al., 1986, 1987) and is also an agonist of imidazoline receptors (Honda et al., 2002). Tizanidine is used for the treatment of spasticity caused by multiple sclerosis (Malanga et al., 2008), neuropathic pain

* Corresponding author.
E-mail addresses: myrnadeciga@hotmail.com, mdeciga@ipn.mx (M. Déciga Campos).

http://dx.doi.org/10.1016/j.ejphar.2017.03.021
Received 26 September 2016; Received in revised form 7 March 2017; Accepted 13 March 2017
Available online 14 March 2017
0014-2999/

(Semenchuk and Sherman, 2000), and phantom limb syndrome (Vorobeĭchik et al., 1997), as well as for acute and intermittent therapy for increased muscle tone (Pappalardo et al., 2006), myofascial pain syndrome (Malanga et al., 2002), and headache (Saper et al., 2002). Tizanidine increases the naproxen´s anti-inflammatory effect (Patiño-Camacho et al., 2013) but not its anti-nociceptive effect when combined with tramadol (Beltrán-Villalobos et al., 2014).

The main result of low doses of this drug combination is the reduction of adverse effects without loss of efficacy. The purpose of this study was to compare the synergistic anti-inflammatory and anti-nociceptive effects of naproxen and ketorolac in combination with tizanidine. To investigate this, we used the carrageenan-induced paw edema as a model of acute inflammation, and the formalin test as a model of nociception. We also demonstrated if the common, adverse effects of ketorolac and naproxen, such as gastric injury, and in the case of tizanidine, a decrement in spontaneous activity, are present in the combinations.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 180–220 g, were used in this study and these animals were provided by the Instituto Nacional de Enfermedades Respiratorias (INER). All experiments were conducted in accordance with the guidelines on ethical standards for the investigation of experimental pain in animals (Zimmermann, 1983) and the protocol was approved by the local institutional animal care and use committees of Mexican regulatory boards: CICUAL from INER, and IPN. The animals were kept under standard laboratory conditions on a 12-h light/dark cycle with light from 7:00 a.m. to 7:00 p.m., and in a temperature-controlled room maintained at (22 ± 1 °C). Animals were housed in individual cages with free access to water. Food was withheld for 12 h before the start of experiments. All experiments were blinded; each rat was used in only one experiment, and at the end of the experiments they were euthanized in a carbon dioxide chamber. For all experimental procedures, groups consisted of at least six rats.

2.2. Chemicals

Naproxen, tizanidine, ketorolac, carboxymethyl cellulose, yohimbine, idazoxan and lambda carrageenan fraction were purchased from Sigma-Aldrich (St. Louis, MO, USA). Formaldehyde solution (37%) for preparation of 1% formalin was purchased from J.T. Baker (Pennsylvania, USA). Naproxen, tizanidine, ketorolac, yohimbine, and idazoxan, were suspended in a vehicle (0.5% carboxymethylcellulose in 0.9% saline solution). Formalin was dissolved in 0.9% saline solution.

2.3. Measurement of inflammation

The carrageenan-induced paw edema model was used as previously described (Patiño-Camacho et al., 2013). All treatments were orally administered to the different groups and immediately before a subcutaneous carrageenan (1%) injection subcutaneously into the right hind paw (50 µl, 10 mg/ml). The volume of the edema (ml) was measured using a plethysmometer (plethysmometer 7150, UGO Basile, Italy). Measurements were at 1, 2, 3, 4, 5, and 6 h, after carrageenan injection to determine differences in paw volume. Inflammation was expressed as the percentage change in paw volume (Patiño-Camacho et al., 2013).

2.4. Measurement of nociception

The nociceptive formalin model was used as previously described (Dubuisson and Dennis, 1977). All treatments were orally administered to the different groups 65 min before subcutaneous 1% formalin injection in the dorsal right hind paw (50 µl, 10 mg/ml). The number of flinches was measured over the course of 1 h. Nociception was expressed as the area under the curve (AUC) of the two phases. For the mechanism of action, yohimbine (3 mg/kg, p.o) and idazoxan (2 mg/kg, p.o) were administered 15 min before the combination with the highest combination doses of tizanidine/naproxen (10.9 mg/kg, p.o.) and tizanidine/ketorolac (1.3 mg/kg, p.o).

2.5. Gastric injury

Gastric injury was assayed, as previously reported, using the nonsteroidal anti-inflammatory drugs to produce ulcers or erosions, and these lesions were considered as 100% injury (Déciga-Campos et al., 2003). Naproxen and ketorolac were orally administered alone or in combination with tizanidine, as described in the study design. Two h after treatments, the rats were killed and their stomachs were removed for analysis. The severity of the gastric lesions was quantified using the following scale: 0, absence of injury; 1, spot ulcer; 2, streak < 1 cm in length < 1 mm in diameter; 3, streak > 1 cm in length < 1 mm in diameter; 4, streak > 1 cm in length > 1 mm in diameter; 5, hematoma similar to that produced by ethanol administration (Aladag et al., 2008). The lesions sustained by the rats in the naproxen and ketorolac groups were considered as 100% gastric injury to compare their respective combinations with tizanidine.

2.6. Spontaneous locomotor activity

Spontaneous locomotor activity is useful for demonstrating a sedative effect through decrements in movement. In this study, 60 min after administration of tizanidine, naproxen, ketorolac or their combinations, the rats were placed one by one in the center of an acrylic cage that was divided into 12 squares (12.5 cm×12.5 cm). The number of squares explored by each rat in a 5 min interval was recorded as its ambulatory activity. After this evaluation, rats were submitted to the formalin test. In addition, other groups of rats with 0.1 mg/kg of tizanidine and the combination of tizanidine/naproxen 5.44 mg/kg, p.o. and tizanidine/naproxen 0.6 mg/kg, p.o. were evaluated 15 min after their oral administration.

2.7. Study design

2.7.1. Individual dose-response curves

To build the dose-response curve (CDR) for the anti-inflammatory and anti-nociceptive effects, groups of at least six rats received orally logarithmic increasing doses of each of the drugs: tizanidine, naproxen, and ketorolac (Table 1). The CDRs were plotted and an effective dose of 40 (ED_{40}) was obtained considering a logarithmic linear effect (Tallarida and Murray, 1987). To examine the combinations of tizanidine/naproxen or tizanidine/ketorolac, we selected ED_{40}, because the maximum anti-inflammatory effect of naproxen or ketorolac was less than 60% and we wanted to reduce the dose to minimize adverse effect.

2.7.2. The dose-response effect of combinations of tizanidine/naproxen and tizanidine/ketorolac

Table 1 includes the dose combinations of tizanidine/naproxen or tizanidine/ketorolac used in this study. These were obtained according to the Tallarida method for isobolographic analysis using ED_{40} (Tallarida et al., 1989). We calculated the theoretical additive concentration (Zadd) using the following equation:

$$Zadd = fA + (1 - f)B,$$

where A is the ED_{40} of tizanidine and B is the ED_{40} of the naproxen or ketorolac. For a 1:1 fixed ratio, f is 0.5 and $(1-f)$ is 0.5. Zadd represents the total additive doses of the drugs, theoretically providing