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Effect of diclofenac sodium on seizures and inflammatory profile induced by kindling seizure model

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ABSTRACT

Epilepsy is a disorder that affects 1–2% of the population and a significant percentage of these patients do not respond to anticonvulsant drugs available in the market suggesting the need to investigate new pharmacological treatments. Several studies have shown that inflammation occurs during epileptogenesis and may contribute to the development and progression of epilepsy, demonstrating increased levels of pro-inflammatory interleukins in animal models and human patients. The objective of this study was to evaluate the effect of non-steroidal anti-inflammatory diclofenac sodium on the severity of seizures and levels of pro-inflammatory interleukins in animals with kindling model induced by PTZ. The kindling model was induced by injections of subconvulsant doses of PTZ (20 mg/kg) in alternated days for 15 days of treatment. The animals were divided into four groups: control group given saline, group treated with diazepam (2 mg/kg) and groups treated with diclofenac sodium (5 and 10 mg/kg). After treatment the open field tests was conducted. The severity of seizures was evaluated by the Racine scale. We evaluated the levels of IL-1β, IL-6 and TNF-α in the blood, hippocampus and cortex of animals. The treatment with diclofenac sodium, in the PTZ induced kindling model, decreased severity of seizures and interleukin-6 and TNF-α levels in the hippocampus of animals treated with doses of 5 and 10 mg/kg. New studies are needed to investigate a new therapeutic approach in the treatment of epilepsy with this anti-inflammatory non-steroidal drug.

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1. Introduction

Epilepsy is a neurological disorder characterized by spontaneous and recurrent seizure episodes (Fisher et al., 2005) that affects between 1–2% of the world population. So far, there is no cure and the treatments with anticonvulsants are not totally efficacious for the different types of epilepsy (López-Hernández et al., 2005). The side effects of tolerance, dependence, risk of death, physical injury (due to the episodes), cerebral damage and induction of psychotic reactions become a problem for clinical treatment. Besides that, there is a significant percentage of patients who do not respond to these medications, therefore, it is necessary to investigate new pharmacological treatments (Kwan and Brodie, 2000; Löscher, 2002).

There is a growing interest in the role of proinflammatory cytokines in the pathogenesis of epilepsy. In physiological conditions, cytokines are found in very low levels on the healthy brain tissue, but elevation in the serum levels of these markers have been reported in neurological diseases as cerebral ischemia, epilepsy, Alzheimer's disease, multiple sclerosis, among others (Rao et al., 2008; Virta et al., 2002; Walker and Sills, 2012). An excessive and unregulated production of cytokines may lead to neuronal degeneration and lead to seizures (Sinha et al., 2008).

Proinflammatory cytokines, such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6), vascular endothelial growth factor and the tumor necrosis factor alpha (TNF-α), as well as the anti-inflammatory cytokine interleukin-10, and related molecules have been described in the central nervous system and plasma of seizure

Abbreviations: IL-1β, interleukin-1 beta; IL-6, interleukin-6; TNF-α, tumor necrosis factor alpha; PTZ, pentylenotetrazole.

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experimental models and in clinical cases of epilepsy (Vezzani, 2005; Vezzani et al., 2011; Ravizza et al., 2011).

Epilepsy is able to increase the levels of cytokines. Patients who have epilepsy that resist to the treatment demonstrate proinflammatory cytokines in the plasma. Proinflammatory cytokines such as interleukin-l beta (IL-1B), tumor necrosis factor alpha (TNF-alpha) and IL-6 have been shown to be over-expressed in brain areas of seizure generation and propagation, prominently by glia and, to a lesser extent, by neurons in experimental seizures models (Vezzani et al., 2008). Studies have demonstrated that the increase in cytokines during brain inflammation or peripherally reduces the threshold for seizures and predisposes epilepsy (Rao et al., 2008; Walker and Sills, 2012).

Some studies strongly suggest the possible role of cyclooxygenase isoenzymes in the pathophysiology of epilepsy and the use of COX-inhibitors as an adjuvant therapy in the treatment of epilepsy (Dhir et al., 2006; Cevik et al., 2015; Moskvin et al., 2006). However, there are no studies investigating the role of diclofenac sodium in the treatment of epilepsy. Diclofenac sodium (sodium-(*o*-((2,6-dichlorophenyl)-amino)-phenyl)-acetate), an NSAID, is characterized by a relatively low molecular weight and has potent anti-inflammatory, analgesic, and antipyretic effects. It inhibits COX, decreases release of arachidonic acid, and increases uptake of arachidonic acid. In clinical practice, DS is widely used for the alleviation of pain, fever, and inflammation associated with arthritis, rheumatoid arthritis, osteoarthritis, acute gout, dysmenorrhoea, and is sometimes used postoperatively (Aygün et al., 2012).

This way, the different findings that show the role of the inflammatory process in the epileptogenic activity suggest that new therapeutical strategies that modulate proinflammatory cytokines response might be used for the treatment of epilepsy. Therefore, the aim of this study was to evaluate the effect of the treatment with the non-steroidal anti-inflammatory drug diclofenac sodium on the intensity of seizures in animals that underwent the kindling model induced by pentylenotetrazole (PTZ). Also, the levels of proinflammatory interleukins IL-1β, TNF-α and IL-6 were evaluated.

2. Material and methods

2.1. Animals

In this study 3-month-old male Wistar rats were used, weighing around 300 g, from the central vivarium at Universidade Federal do Rio Grande do Sul. The animals had free access to water and food and were kept in polypropylene boxes that measured $41 \times 34 \times 16$ cm. There were a maximum of 5 animals per box, in a 12-h bright-dark environment at 23 ± 1 °C. All experimental procedures were conducted in the light phase of the light/dark cycle. The experiments were performed in accordance with the “Guide for the Care and Use of Laboratory Animals, DHEW, publication no (NIH) 80-23, 1985” and approved by the local ethical committee at Universidade Federal do Rio Grande do Sul.

2.2. Experimental groups

Four experimental groups were evaluated (10 in each group): positive control group (they received diazepam at a 2 mg/kg dose), negative control group (they received sodium chloride 0,9g%), diclofenac sodium 5 group (they received diclofenac sodium at a 5 mg/kg dose), diclofenac sodium 10 group (they received diclofenac sodium at a 10 mg/kg dose). The animals received daily doses of intraperitoneal (i.p.) diclofenac sodium, diazepam and sodium chloride for 15 days.

2.3. Kindling model

The kindling model, which is considered a chronic model of epilepsy, was used based on Gupta et al. (2003). The animals received the same doses described in the treatments for 15 days, and every other day they also received subconvulsants doses of PTZ intraperitoneally (20 mg/kg of body weight). PTZ was administered 30 min after the administration of the treatments and the animals were observed for 30 min assessing seizure severity using Racine’s adapted scale (Racine et al., 1972).

2.4. Behavior test

After the first day of treatment with the different drug doses, before the induction of kindling, the animals behavioral patterns were assessed, using the Open Field. For the Open Field test the rats were placed in an acrylic box, whose lower part was divided by black lines in 12 same-sized quadrants, the rats were left to explore the box freely for five minutes.

The open field apparatus was a 40 cm × 60 cm linoleum floor surrounded on three sides by a 60 cm-high plywood wall and in the front by a 60 cm-high glass wall. Crossings of the lines (locomotion and exploration), rearing responses (orienting-investigatory responses), grooming and fecal bolus (anxiety manifestation) were analyzed for each rat (Izquierdo, 1989). After each test, the floor was cleaned with water and ethanol to avoid a potential excitatory effect on locomotion produced by the presence of urine residues.

2.5. Brain microdissection and tissue preparation

At the end of the study, the animals were sacrificed by decapitation and the brains were immediately removed and kept on an ice-plate. Cerebral areas cortex and hippocampus were dissected and kept chilled until homogenization. The brain tissues were homogenized in 1:5 (w/v) saline solution (0.9% NaCl). The homogenate was centrifuged at $800 \times g$ for 10 min at 4 °C, and the supernatant was used in the assays.

For the acquisition of serum, the whole blood was centrifuged at $1,000 \times g$ for 5 min, and serum was immediately removed. Samples were stored at −80° C until further analysis.

2.6. Cytokines determination

TNF-alpha, IL-1β and IL-6 were measured by enzyme linked immunosorbent assay (ELISA) kits (Abcam, Cambridge, USA) in serum, hippocampus and cerebral cortex. The assay utilizes ELISA strip plates pre-coated with a capture monoclonal antibody (mAb), to which samples are added. Captured cytokine is detected by adding a biotinylated mAb followed by streptavidin-horseradish peroxidase. Addition of the enzyme substrate TMB results in a colored substrate product. Intensity of the color is directly proportional to the concentration of cytokine in the sample, which is determined by comparison with a serial dilution of recombinant cytokine standard analyzed in parallel. All samples were run in duplicate. The readings were conducted in an ELISA reader (Biochrom Anthos Zenyth 200rt) at 450 nm. Concentration was calculated using standard curve from different concentrations of recombinant cytokine, as per the manufacturer’s specifications. The results were expressed as pg/mL for serum and pg/mg of protein for brain tissues.

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