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Inflammatory pain assessment in the arthritis of the temporomandibular joint in rats: A comparison between two phlogistic agents

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ABSTRACT

Temporomandibular joint (TMJ) disorders are a group of conditions that result in TMJ pain, which frequently limits basic daily activities. Experimental models that allow the study of the mechanisms underlying these inflammatory and pain conditions are of great clinical relevance. The aim of this study was to evaluate nociception, inflammation and participation of the macrophage/microglia cells in the arthritis of the TMJ induced by two phlogistic agents. 84 rats were divided into 2 groups: Zy, which received zymosan intra-articularly, or Cg, which received carrageenan intra-articularly. Mechanical nociception, total leukocyte influx to the synovial fluid and histopathological analyses were evaluated in the TMJ. The participation of macrophage/microglia located in trigeminal ganglia (TG) and in the subnucleus caudalis (V-SnC) was assessed immunohistochemically. Both agents induced mechanical hyperalgesia 6 h after the induction, but a more persistent algesic state was perceived in the Cg group, which lasted for 120 h. Even though both groups presented increased leukocyte influx, the Zy-group presented a more intense influx. Zymosan recruited resident macrophage in the trigeminal ganglia 24 h after the injection. In the V-SnC, the group Cg presented a more prolonged immunolabeling pattern in comparison with the group Zy. It can be concluded that zymosan induced a more intense infiltrate and peripheral nervous changes, while Cg lead to a moderate TMJ inflammation with prominent changes in the V-SnC.

1. Introduction

Disorders regarding orofacial pain are debilitating conditions that affect the head, face and/or neck region of around 90% of the population and might have physical or psychological origins (Romero-Reyes & Uyanik, 2014). With the exception of tooth-related pain, temporomandibular joint (TMJ) disorders are the main reason that lead people to seek treatment for orofacial pain, even though only 10–20% of those patients are properly treated (Nassif, Al-Salleeh, & Al-Admawi, 2003; Von Korff, Dworkin, Le Resche, & Kruger, 1988).

Regardless of the nature of the orofacial pain, a general mechanism for the conduction of a nociceptive impulse might be explained. A local noxious stimulus depolarizes the peripheral free nerve endings of a primary neuron and the action potential conducted to the subnucleus caudalis (V-SnC), where the first synapse takes place. Consequently, the impulse is finally conducted to the ventral posteromedial nucleus of the thalamus and to the somatosensory cortex (Takemura, Sugiyo, Moritani, Kobayashi, & Yonehara, 2006).

For an extensive period of time, the physiopathology of pain relied mainly on the synaptic role of the neuron (Hucho & Levine, 2007). However, it is now clear that macrophage/microglia cells exert an important function within the pain process, modulating neuronal synaptic function and neuronal excitability by various mechanisms (Halassa, Fellin, Takano, Dong, & Haydon, 2007). Astrocytes, microglia and satellite glial cells were shown to be capable of changing the production of cytokines and chemokines in degenerative inflammatory conditions (Souza et al., 2013; Suter, Wen, Decosterd, & Ji, 2007) and microglial cells, different from other mononuclear macrophages associated to the central nervous system (CNS), interact with neurons, synapses and other glial cells in pathological conditions in the CNS parenchyma (Mosser, Baptista, Arnoux, & Audinat, 2017). Microglial activation increases proliferation, phagocytic activity and the release of pro-inflammatory mediators, which activate the neurons in an individualized manner, favoring nociception (Milligan & Watkins, 2009). It was shown that TMJ inflammation induced by Complete Freund's Adjuvant (CFA) activates glial and immune cells in both the TG and in

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the V-SnC (Villa et al., 2010). Evidence suggests that glial cells of sensory ganglia also participate in the development and maintenance of chronic pain condition (McMahon & Malcangio, 2009).

Even though several experimental models are used for studying disorders of the TMJ, little is known about the nociceptive mechanism and the changes in the central and peripheral nervous systems among the different models. A proper study of the pathogenesis of induced arthritis models is then indicated in order to elucidate the choice of the most accurate experimental model depending on the aims of the study. Therefore, the purpose of this study was to evaluate the nociception, inflammation and the participation of macrophage/microglial cells in two different models of TMJ arthritis.

2. Materials and methods

2.1. Sample size

For calculating the sample size, Wistar rats (180–200 g) were considered as the study unit and the head withdrawal threshold was considered the primary outcome. The sample size was determined in order to recognize a significant difference of 20% from the baseline in the primary outcome with a power of 80%. The standard deviation was established at 15% with a 95% interval of confidence. Therefore, a sample size of 7 animals was required for each experimental group.

2.2. Animals

The experimental protocols for this study was approved and analyzed by the Ethics Committee on Animal Research at Federal University of Ceará - UFC (protocol 27/10) and considered in compliance with the ethical principles of animal experimentation, as well as standards for the didactic-scientific practice of vivisection and the National Institutes of Health guide for the care and use of laboratory animals

Eighty-four adult male rats (*Rattus norvegicus*, *albinus*, Wistar), weighing between 180 g and 200 g, were provided by the Animals Facility in the Department of Physiology and Pharmacology of the Federal University of Ceará. The animals were housed in proper plastic cages, with no > 6 animals per cage, with solid food and water *ad libitum* in a room with a 12 h light/dark cycle at a temperature of 22 ± 2 °C.

The animals were then divided into 2 major groups: group Zy, which received a 40 μl intra-articular injection of a 5% zymosan solution, and group Cg, which received a 10 μl intra-articular injection of a 5% carrageenan solution, following well established models for TMJ arthritis (Cavalcante et al., 2013; Chaves et al., 2011).

2.3. Induction of the TMJ arthritis

The animals were anesthetized by an intraperitoneal injection of a solution of Ketamine (70 mg/kg) and Xylazine (10 mg/kg) and received an intra-articular (i.art.) injection of zymosan (2 mg, 40 μl total volume) or 5% Cg solution (500 μg per articulation;10 μl) dissolved in sterile saline into the supra-discal space of the left TMJ (ipsilateral) using a microsyringe (Hamilton model 705RN; Hamilton, Reno, NV, USA) coupled to a 30-gauge needle (BD, Franklin Lakes, NJ, USA). As a control procedure, another group of animals was intraarticularly injected with saline unilaterally in the left joint.

In order to properly locate the TMJ capsule, the animals' mandibles were manipulated and the needle was inferiorly inserted to the posterior inferior border of the zygomatic arch. When a resistance was found, the mandible was once again manipulated to confirm the capsule location. The accuracy of the injection was confirmed by the lack of resistance when the needle passed through the capsule (Chaves et al., 2011; Gondim et al., 2012).

2.4. Evaluation of the mechanical sensitivity

The nociceptive threshold of the animals was assessed by the intensity of pressure applied to the left TMJ area that generated a reflex response (head withdrawal threshold; HWT), using an electronic Von Frey equipment (Analgesímetro Digital, Insight, Ribeirão Preto, SP, Brazil). The facial areas to be tested around the TMJ were carefully shaved, and the animals were placed into individual plastic cages 45 min before beginning the tests. During the 5 days preceding the experimental period, the animals were adapted for manipulation in order to properly assess their nociceptive threshold without stressing the animals. The transducer was perpendicularly applied to the central area of the TMJ region with a gradual increase in pressure. The stimulus was automatically discontinued, and the intensity was recorded when the head was withdrawn. The end-point was characterized by the removal of the head in a clear flinch response after head withdrawal (Denadai-Souza et al., 2010).

In both groups, the HWT was measured before the induction of the arthritis of the TMJ and 6, 24, 48, 72 and 120 h after the administration of the phlogistic agents.

2.5. Total leukocyte count in the synovial fluid

Our group has previously shown that the 6th hour is marked by an intense inflammatory infiltrate followed by the injection of both Zy and Cg (Cavalcante et al., 2013; Chaves et al., 2011). Therefore, 6 h after the induction of TMJ arthritis, a subgroup of the animals was euthanized by intracardiac perfusion with 40 ml of saline solution followed by 40 ml of 4% paraformaldehyde (PFA). The skin and the temporal muscle were deflected and the articular wash was performed by injecting and aspirating 100 μl (2 × 50 μl) of saline solution with heparin (5 U/ml) using ultrafine insulin syringes. A sample of 20 μl from the articular wash was mixed with 380 μl of Turk's solution and the cells were counted under light microscopy with a Newbauer's Chamber.

The synovial fluid of the TMJ of saline group was collected, following the same procedures, in order to compare the leukocyte count within these animals with the animals treated with the phlogistic agents.

2.6. Histopathological analysis

A histopathological analysis of the TMJs was performed in the peak of pain for each model. The rats were euthanized, the facial skin was excised, and the temporal muscle that overlays the TMJ was dissected. After the TMJ and periarticular tissue were excised, the tissues were fixed in 10% neutral buffered formalin for 24 h, demineralized in 10% ethylenediaminetetraacetic acid, embedded in paraffin and sectioned along the long axis of the TMJ. Sections (4 μm) showing the mandibular condyle, articular cartilage, articular disc, synovial membrane, periarticular tissue, and skeletal muscle periarticular tissue were evaluated under light microscopy by a certified histotechnologist (G.A.B.), who evaluated the inflammatory infiltrate influx, as well as the integrity of the articular tissues (synovial membrane, mandible condyle and articular cartilage).

2.7. Immunofluorescence analysis of ionized calcium binding adaptor molecule-1 (Iba-1) expression in the trigeminal ganglion

After the animals were euthanized, their brains were removed and the left trigeminal ganglia were easily seen in the intern aspect of the neurocranium. The ipsilateral TG was removed and fixed for 2 h in 4% PFA. It was then cryoprotected in 30% sucrose solution for 72 h, embedded in Tissue-Tek® and froze-stored at − 80 °C until further evaluation. Sixteen μm-thick histological sections were mounted in poly-L-lysine microscope slides and went through antigenic retrieval in citrate