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Chronic stress effects in contralateral medial pterygoid muscle of rats with occlusion alteration

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HIGHLIGHTS

- Occlusion alteration produce metabolic changes and oxidative stress in masticatory muscle.
- Chronic stress induces an adaptation of the muscle contralateral to the dental extraction.
- There is a potential link between stress state and muscle alteration in TMD.

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ABSTRACT

Temporomandibular disorder (TMD) has a high prevalence in our society, characterized by a severe pain condition of the masticatory muscles and temporomandibular joint. Despite the indication of multiple factor initiators of TMD, there is still controversy about its etiology and its pathophysiology is poorly understood. Using rats as experimental animals we investigated the effect of unpredictable chronic stress with or without unilateral molar extraction on the contralateral medial pterygoid muscle. Our hypothesis is that these two factors induce changes in morphology, oxidative metabolism and oxidative stress of muscle fibers. Young adult male Wistar rats (±200 g) were divided into four groups: a group with extraction and unpredictable chronic stress (E + US); with extraction and without stress (E + C); without extraction and with unpredictable chronic stress (NO + US); and a control group without either extraction or stress (NO + C). The animals were subjected to unilateral extraction of the upper left molars, under intraperitoneal anesthesia with 4% Xylazine (10 mg/kg) and 10% Ketamine (80 mg/kg) on day zero. The rats of groups E + US and NO + US were submitted to different protocols of stress, from the 14th day after the extraction. The protocols were different every day for five consecutive days, which were repeated from the 6th day for five days more. Contralateral medial pterygoid muscles were obtained on the 24th day after the start of the experiment for morphological, metabolic, capillary density, and oxidative stress analysis. The data from capillary density showed a decrease of capillaries in animals subjected to dental extraction, compared with those without extraction and an increase of laminin expression in the group submitted to the unpredictable chronic stress when compared to the unexposed to stress. SDH test revealed a decrease of light fibers in the group submitted to unilateral extraction of molars, compared with this area in the control group. In E + US and NO + US groups, the deeply stained fibers increased compared to NO + C. The exodontia factor was able to increase the ROS activity in muscle, whereas the stress factor does not significantly alter ROS in this tissue. It was concluded that both unpredictable chronic stress and the extraction induce metabolic and density of capillary changes in the contralateral medial pterygoid muscle to extraction, suggesting that these factors for a longer period of this experiment could induce muscle damage related to TMD.

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1. Introduction

Among the functional disorders that affect the stomatognathic system, temporomandibular disorder (TMD) associated with myofascial pain has a high prevalence in our society. This dysfunction is characterized by a severe pain condition or malfunction of muscles of mastication and/or temporomandibular joint [1]. Temporomandibular disorder has a considerable effect on work, family life and social activities of the patient [2], leading to a large percentage of these patients to seek appropriate treatment. However, TMD associated with myofascial pain still is controversial as to its etiology [3] and the treatment is still empirical [4] due to few scientific studies demonstrating the efficacy of pharmacotherapy.

It is well established that musculoskeletal disorders comprise one of the most common and costly public health problems. Orofacial myopathy seems to be a consequence of an increase in sustained muscle activity during exposure to stressors that over time may lead to muscle overuse, damage, and subsequent musculoskeletal pain [5]. The repetitive muscle contractions lead to a variety of phenotypic and physiological responses including activation of mitochondrial biogenesis, fiber type transformation and angiogenesis [6].

According to Okeson [7], the emotional stress related to bruxism, leads to increased muscular tone and develops the non-functional muscle activity of head and neck muscles. In the same context, Rosales et al. [8] have shown some findings that suggest that emotional stress induces bruxism-like activity in the masseter muscle of rats, which was reduced with anti-anxiety drugs. Also, physiologic and hemodynamic changes were found in masticatory muscles induced by emotional stress [9]. The masticatory muscle disorders may also be related with malocclusion [10,11] and postural change. Despite the indication of a multifactorial etiological relationship of TMD, there is still controversy about its etiology, and TMD pathophysiology is poorly understood.

The literature reveals various methods used for the analysis of the masticatory muscles under functional modifications such as painful mechanisms [12], histoenzymology [13] and microscopic analysis [14]. In this context, the response to enzyme succinate dehydrogenase (SDH) demonstrates oxidative capacity and muscle metabolic activity. In an SDH reaction, the tetrazolium nitroblue acts as a hydrogen acceptor, which upon contact with the substrate forms the product of this blue color reaction, which enables muscle analysis according to the intensity of staining muscle in light, intermediate and dark fibers [15]. In addition, a smaller number of studies have examined the extracellular component by immunohistochemistry [16]. Laminin is an immunostainer used to identify endothelium in microvessels and capillaries [17]; to precisely map the binding site responsible for mediating the interaction on the surface of endothelial cells [18]; or to study capillary density as a result of oxidative capacity in muscle [19,20]. Immunohistochemistry for reactive oxygen species (ROS) in turn, allows us to evaluate reactive derivatives of oxygen metabolism, which in physiological conditions are involved in intracellular signaling and in regulating gene transcription and protein synthesis processes [21]. The acute effect of ROS on muscle function mediated by changes in the sensitivity of myofibrillar Ca^{2+} may contribute to the development of muscle fatigue [22], however, the mechanism of action of ROS is still poorly understood [23].

As the cellular and molecular changes in the muscles subjected to altered occlusion and stress remain largely unexplored, the purpose of this study was to use some of these methodologies to provide data that could contribute to the understanding of TMD and myofascial pain. Our hypothesis is that the stressors and teeth extraction alter the activity of the muscles of mastication. Therefore, in this study we assessed the metabolic activity, capillary density, and ROS activity in contralateral medial pterygoid muscles of rats subjected to unilateral extraction with or without the unpredictable chronic stress.

2. Methods

2.1. Animals

Experiments were performed with male Wistar rats weighing 275 ± 300 g, obtained from the University of São Paulo, Campus of RibeirãoPreto, Brazil. The animals were housed in a temperature controlled room (24 ± 1 °C) and 12 h light/dark cycles (lights on at 06:00) with food and water ad libitum. All experimental procedures were approved by the Animal Use and Ethics Committee of RibeirãoPreto, University of São Paulo (Process number 12.1.418.53.0). All efforts were made to minimize animal suffering and to reduce the number of animals used.

The animals were randomly divided into four groups ($n = 8$ per group): a group with extraction and unpredictable chronic stress (E + US); with extraction and without stress (E + C); without extraction and with unpredictable chronic stress (NO + US); and a control group without both extraction and stress (NO + C).

2.2. Induction of modified occlusion by unilateral edentulism

On day zero, the rats were subjected to the extraction of the upper left molars. The animals were anesthetized with an association of 4% Xylazine (10 mg/kg) and Ketamine (80 mg/kg). The extractions were performed by using an anatomical clamp and the Hollemback 3S (child), disinfected with iodine solution. As a prophylactic measure, the animals received a single dose of an antibiotic (Pentabiotic veterinary - "Fort Dodge") in a dose of 24 mL of penicillin per kg of body weight, as well as anti-inflammatory and analgesic Bananine (Schering-Plorigh, flunixin meglumine, 25 mg/kg, 10 mg/mL). Considering the surgical stress induced by the extraction, in the NO groups a simulation of the extraction process was conducted, so the rats were anesthetized and received the same dose of antibiotic and anti-inflammatory treatments.

There were not used sutures after molars extraction. On the 14th day, visually, it is possible to verify complete healing of the extraction region. Also, the socket is almost totally occupied by reticular bone and the trabecular surfaces show intense bone activity in this day. Besides that, the wound surface is completely covered by new epithelium.

2.3. Chronic stress protocol

This protocol consisted of ten consecutive days of stress beginning on day 14 (14 days after molars exodontia or simulation).

Unpredictable chronic stress protocol in the E + US and NO + US groups was performed using five different methodologies in a ten-day period (14th to 23rd day). Stressful situations were started at 9:00 AM.

- Day 01 and Day 06: Agitation. Rats were individually placed in a plastic box on a shaker table for 15 min. The average speed of rotation was 50 rpm.
- Day 02 and Day 07: Forced Swim. Rats swam for 15 min in a plastic circular container with a depth of 54 cm and a diameter of 47 cm, filled with water to a depth of 40 cm, preventing contact with the floor or upper edge.
- Day 03 and Day 08: Physical Restraint. The animals were placed in a metal box, which was 15 cm long and 5 cm in diameter and had adequate ventilation throughout its length. The end of the box was closed, and the animals were in a state of physical restraint for 2 h, which restricted their movements.
- Day 04 and Day 09: Cold stress. Rats located in individual plastic boxes were exposed to hypothermia in freezer (4 °C), for a period of 30 min.