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Research Article

Histological muscle evaluation in experimental animal model after inducing occlusal interference

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Abstract. Objective. The occlusal contact patterns of the teeth will influence the precise functional activity of the masticatory muscles. Occlusal interferences are associated, in the short term, with clinical signs and symptoms, such as localized pain, in the masticatory muscles. The aim of this study is to evaluate the histological changes in the masseter muscle following occlusion changes by inducing interferences in rats.
Material and methods. Twenty Wistar rats were randomized into three experimental groups and one control group (5 rats per group). Animals in the experimental group were cemented modified crowns on their mandibular first molars to induce occlusal trauma in 7, 14 and 30 days.
Results. Most of the muscle fibers in experimental rats remained intact, although partial histological changes, such as: multiplication of connective tissue, the appearance of mononuclear inflammatory cells. The results showed that there was a statistically significant relationship between the group with occlusal trauma for a period of 30 days (p =0.026) and the control group, concerning the diameter of the muscle fibers.
Conclusion. These results showed that occlusal interference caused histological changes in masseter muscles and that this may be related to the fact that occlusal changes reduced the energy level in the masseter muscles while performing mastication movements as a result of the decrease in the oxidative metabolism.

Key Words: occlusal interference, masseter muscle, mechanical hyperalgesia.

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Introduction

The occlusal contact patterns of the teeth will influence the precise functional activity of the masticatory muscles (Okeson 2013). Some studies (Solberg et al 1979; Ericksson et al 1983; Kirveskari et al 1992; Tolska et al 1995; Okeson 2013) reveal a positive relationship beetwen occlusal factors and masticatory symtoms, while others (Vanderas 1994; Seligman & Pullinger 1991) show no relationship. It has been demonstrated that specific occlusal contact patterns can influence specific muscle groups (Williamson & Lundquist 1983; Belser & Hannam 1985; Okeson 2013).

Occlusal interferences are associated, in the short term, with clinical signs and symptoms, such as localized pain, in the masticatory muscles (Popa 2004; Okeson 2013; Liu et al 2013). Thus, in the lateral teeth, they produce inhibition of the normal reflex of reducing the contraction in the elevator muscles. They trigger synchronized and disorganized pulses initiated by the proprioceptive receptors located in the periodontal ligaments of the interference teeth (Popa 2013).

Interferences induce and maintain an abnormal muscle activity in the contractions of the masticatory muscles, which become hypertonic and disorganized. Hypertonic and synchronous contractions can generate exaggerated contractions in the masticatory muscles before the mandible being correctly positioned by the temporal muscles. This type of asynchronous contraction generates pathological occlusal forces targeted at the contact teeth. The aim of this study is to evaluate the histological changes in the masseter muscle following occlusion changes by inducing interferences in rats. The main objective of the present study was to highlight the structural changes in the deep masseter muscles in the experimentally induced occlusal trauma (OT). On the muscle sections, the transverse diameter of the muscle fibers was measured in the deep masseter muscles.

Materials and methods

In view of this study, we designed an animal model and carried it out in the Department of Physiology at "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca.

In the present study we included 20 female rats, Wistar white (average weight 300 g), distributed randomly into four groups (n = 5). The animals were housed in type II L polycarbonate cages, in a room with a constant environmental temperature (21.5 to 23 degrees) and a relative humidity of 65%. The animals were exposed to a standard 12-hour light/dark cycle. The rats were fed ad libitum on a light diet and water. The research protocol was approved by the Ethics Committee of UMF Cluj-Napoca and was registered under the number 39/16.

Animals were distributed randomly into 4 groups as follows:

1 - control group
2 - group with occlusal trauma for a period of 7 days
3 - group with occlusal trauma for a period of 14 days
4 - group with occlusal trauma for a period of 30 days
Occlusal interference induction was obtained by applying 0.5 mm - thick Ni-Cr metal crowns on the first mandibular molar in the right side. The rats were anesthetized intramuscularly with 0.3 mg/g body weight ketamine and with 0.1 mg / g body weight narcoxyl. Before applying the metal crown, the occlusal surface underwent professional brushing. The crowns were cemented with dual cement - Bis Cem.
After 7, 14, 30 days of the bite raise, the animals were anesthetized and euthanized. Anesthesia was performed by intramuscular injection of 0.3mg/g body weight ketamine and 0.1 mg / g body weight narcoxyl.
For cementation, the samples were placed in 10% neutral, buffered formalin solution. Next, the samples were decalcified by immersing the ends, for 48 hours, in a mixture of 8% formic acid and 8% hydrochloric acid.
After cementation and decalcification, the samples were processed by the paraffin technique in view of performing the histopathological examination. Sections were made in the deep transverse and longitudinal masseter muscles.
We made serial 4-micrometer thick by using the Leica RM 2125 RT microtome, and the sections were laid on ordinary histological smears for the histopathological examination to be performed by hematoxylin-eosin staining.
The protocol guiding the hematoxylin and eosin (H & E) staining was as follows: dewaxing the samples by immersion into two xylene baths for 3 minutes, hydrating the samples in three successive ethanol baths (100% - 3 minutes, 90% - 3 minutes and 70% - 3 minutes) followed by immersion into distilled water.
The next immersion was into Gill 2 hematoxylin for 2 minutes, then, washing in a bath with tap running water for 3 minutes, followed by immersion into a eosin-floxin solution for one minute and rinsing with tap water. This was followed by dehydration of the samples in three successive ethanol baths (70% - 3 minutes, 90% - 3 minutes and 100% - 3 minutes), then clarification by double immersion into xylene for 3 minutes, followed by cementation and a final examination under the microscope.
The preparations were examined using an Olympus BX 51 microscope whereas the images were taken with an Olympus UC 30 digital camera and processed by the Olympus Stream Basic image acquisition and processing program.
We performed a morphometric examination for measuring the diameter of the muscle fibers in the deep masseter muscles in three different sites.
Statistical analysis was carried out using the MedCalc Statistical Software version 17.6 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017). Data were analyzed using the t test for independent variables and the paired t test. A p value <0.05 was considered statistically significant.

Results

In the animals of the control group, the muscle fibers in the masseter muscle were found to be heterogeneous in terms of size. No degenerative fibers were identified. Cell nuclei were observed throughout the periphery of the muscle fibers (Fig. 1).

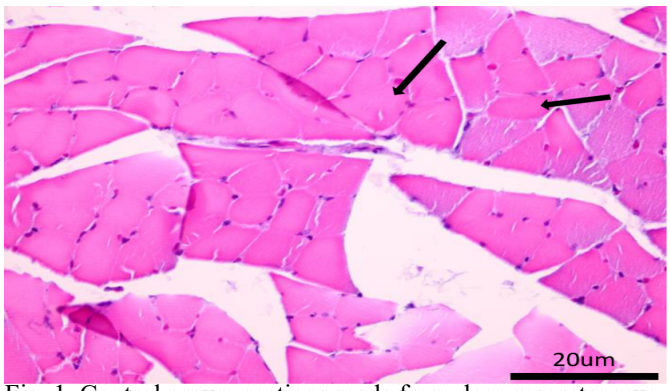

Fig. 1. Control group, sections made from deep masseter muscles, heterogeneity of muscle fibers (arrows), HE x400

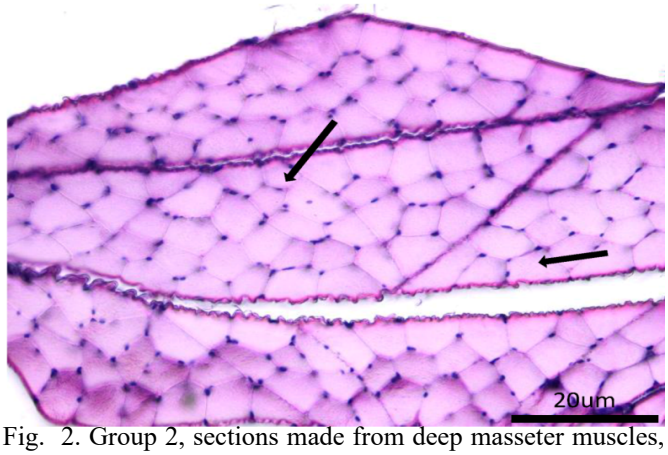

Fig. 2. Group 2, sections made from deep masseter muscles, muscle fibers of different shapes and diameters (arrows), HE x400

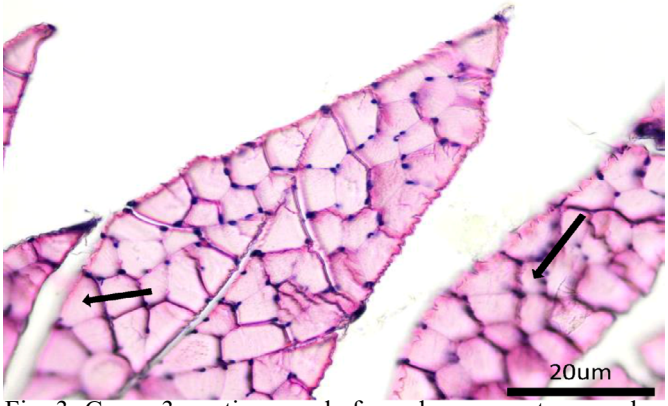

Fig. 3. Group 3, sections made from deep masseter muscles, heterogeneous muscle fibers, HE x400

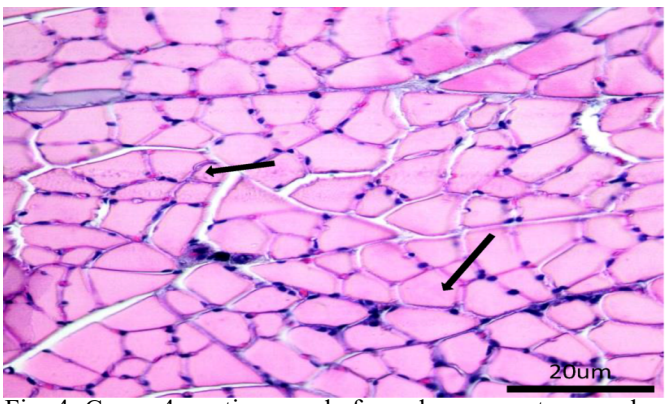

Fig. 4. Group 4, sections made from deep masseter muscles, heterogeneous muscle fibers, different shapes and diameters (arrows), HE x400

No histological changes were identified in the animals from group 2 as compared to the animals in group 1 (Fig. 2).
Partial histological changes, such as: multiplication of connective tissue, the appearance of mononuclear inflammatory cells in the muscle fibers were noticed in the animals from group 3, (Fig. 3). A small number of fibers with central nuclei were also identified, which showed a loss of mitochondria.
A large number of inflammatory cells in the muscle fibers was observed in the group 4 animals, which suffered occlusal trauma for 30 days as compared to those in group 3, which suffered occlusal trauma for 14 days. In addition, a large number of fibers with central nuclei in the regenerated fibers was identified (Fig. 4).
The histometric analysis of masseter muscles results are shown in table no 1.

Table 1. The histometric analysis of masticatory masseter muscles fibres in experimental animals

Group of animals included in the study	Mean	Std. Deviation	p
Control group	23.33	5.5	-
Group 2 (OT=7 days)	2124	5.2	0.2
Group 3(OT=14 days)	23.52	4.9	0.6
Group 4 (OT=30 days)	9	1	0.02

Regarding the histometric analysis of masticatory masseter muscles, the results showed that there was statistically significant relationship between control group and group with occlusal trauma for a period of 30 days, (p=0.02), but the relationships between control group and occlusal trauma for a period of 7 days or 14 days were not statistically significant (p >0.05).

Discussions

The study results showed that the occlusal interference led to relevant histological changes in the masseter muscles, similar to those reported by other authors (Nishide et al 2001; Cao et al 2009) on the histological changes in the masseter muscles in rats, following the experimental changes of the occlusion by occlusal raise.
Researchers of a recent study reported that the mastication force increased in rabbits in a reflex way when a 2-mm-thick film of polyurethane was applied between the upper and lower molars, and the occlusal force increased in direct proportion to the hardness of the film interposed. The results are similar to those shown in the present study on the experimental model in rats (Morita et al 2015).
These results confirm that the presence of supraocclusal contacts increase the activity in the masseter of the active side when the food is crushed, whereas the contacts of the balance side can induce easy reflex opening of the buccal cavity due to the sensorial proprioceptive impulses triggered from the periodontal membrane (Hidaka et al 1997).
Such views have been in keeping with other authors who maintain that the increased activity of the masseter muscle depends on where the occlusion is raised (Morita et al 2016).
A study carried out on Wistar rats confirms the histological changes produced in the masticatory muscles following a 1-2 mm increase of the vertical dimension of the occlusion. Tissue reactions, starting from the acute stage of the inflammation resulting in the destruction of myofibrils up to the healing stage with myofibrillar regeneration, were much more evident in the deep masseters, when the bite was raised by 2 mm. In addition, a transient inflammation of the superficial masseters in the rats, with a 1-mm raise of the occlusion was reported (Akagawa et al 1993).
Four weeks of the raise, the masseter muscles were removed in order to identify the histological changes. The results showed that the occlusal interference induced histological changes such as the appearance of inflammatory cells and muscle fibers with central nuclei. The present study also revealed the fact that occlusal changes reduced the energy level in the masseter muscles while performing mastication movements as a result of the decrease in the oxidative metabolism.
The central sensory nervous response has an important role in causing muscle pain induced by occlusal trauma (Nishida 1997). It is possible that bite raising affects masseter muscle activity, because the activity of the jaw closing muscles is modulated by sensory afferents from the muscle spindles.
Morita et al (2016) reported that the muscle activity of the masseter muscles intensified by raising the bite in the active side in half of the animals included in the study. The modulation of the activity in the masseter muscle appears to depend on the side where the raise of the occlusion is implemented rather than on the degree of the raise.

Conclusion

These results showed that occlusal interference caused histological changes in masseter muscles and that this may be related to the fact that occlusal changes reduced the energy level in the masseter muscles while performing mastication movements as a result of the decrease in the oxidative metabolism.

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