

# Clinical and Histochemical Alterations of the Periodontal Ligament in Gerbils after Malocclusion Induced

## Alteraciones Clínicas e Histoquímicas del Ligamento Periodontal en Gerbiles Después de Maloclusión Inducida

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**SUMMARY:** The aim of this article is to show the clinical and histochemical alterations of the first periodontal ligament, on the right side, after upper molars teeth extraction on the left side in gerbils. After two months, the periodontal ligaments were removed and processed for histochemical analysis. The data showed that TRAP reaction was able to evidence the osteoclastic activity in the hyperfunction hemimandible, right side, explaining the functional changes in the periodontal ligament after teeth extraction, and a little gingival recession and radicular exposure of teeth without function was observed at inferior molars of the left side.

**KEY WORDS.** Periodontal ligament; Histochemistry; Malocclusion, Gerbil; *Meriones unguiculatus*.

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### INTRODUCTION

The periodontal ligament is the connective tissue localized between the root of the tooth and the alveolar bone, and is known to aid in relieving mechanical strain such as occlusal force and orthodontic force (Shuttleworth & Smalley, 1983; Kusters *et al.*, 1991; Kuroiwa *et al.*, 1992).

The periodontal ligament consists of cellular and intercellular components, which fills up the space around cells. Intercellular components are mainly composed of fibrous elements and the ground substance. The major fibrous elements are collagen fibers (type I and type III collagens), which play a role in resisting tensional force and holding teeth in the alveolar socket (Shuttleworth & Smalley; Kusters *et al.*).

Hypofunction such as a loss of the opposite tooth results in disuse atrophy, as observed in various organs. Although the periodontal principal fibers are disorganized at various degrees, disappear, and are replaced by a loose connective tissue under hypofunctional conditions, this atrophic phenomenon is reversed by recovery of the

masticatory function (Cohn, 1965; Amemiya & Abe, 1980; Kinoshita *et al.*, 1982; Koike, 1996).

Tartrate-sensitive acid phosphatase has been cytochemically localized in osteoblasts, osteocytes, and osteoclasts, whereas tartrate-resistant acid phosphatase (TRAP) is present in osteoclasts (Hammarstrom *et al.*, 1983; Minkin, 1982). Osteoclasts have been identified, primarily by morphological criteria, as large, multi-nucleated cells which contact calcified bone matrix and exhibit ruffled borders surrounded by clear zones (Gothlin & Ericsson, 1976). Tartrate-resistant acid phosphatase as a histochemical and biochemical marker for the osteoclast would provide an additional experimental tool for studying osteoclastic differentiation and function. Baron *et al.*, (1986) employed TRAP as such a marker for cytochemical identification of proposed osteoclast precursors at both the light and the electron microscopic level.

Thus, the objective of this study is to show the clinical and histochemical alterations of the periodontal ligament in

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the first molar, on the right side, after upper molar teeth extraction on the left side in gerbils.

## MATERIAL AND METHOD

This study followed the requirements of the Ethics Committee on the Use of Animals in Experimentation at the University of São Paulo, Brazil.

It was used 10 adult male gerbils (*Meriones unguiculatus*), weighing 50g, maintained at the animal house under controlled temperature, with commercial ration and water available at libitum.

A half of these animals received the occlusal alteration induction by upper molars teeth extraction (Fig 1A), on the left side, experimental group, and the other half was considered as control, only submitted to surgical stress. These animals were used for histochemical study.

**Induction of occlusal alteration.** The animals were anesthetized with tribromoethanol (0.25g/kg of body weight) and submitted to teeth extraction of the upper molars in the left hemiarch, with animals receiving the antibiotic Pentabiotic (24000 IU for kg of body weight) as preoperative prophylaxis. After asepsis and disinfection of the surgical site, teeth extraction was performed with anatomical tweezers and hollenback 3S. The extraction area was compressed to stop bleeding (Chompret maneuver). After surgery, the animals received antiinflammatory and analgesic drugs (sodium diclofenac- 0.4mg/Kg of body weight) and were maintained in appropriate cages for two months.

**Specimen preparation for histochemical analysis.** After two months, the animals were anesthetized with urethane (1.5g/kg of body weight) and injected intracardially with 7% of body weight 0.9% physiological saline to wash the vessels, followed by perfusion with the same amount of 10% formalin in sodium phosphate buffer, pH 7.4. The hemimandibles with periodontal ligament were then removed and immersed in the same fixative solution. After fixation period, the specimens were washed in cacodilat solution and dried and decalcified in EDTA 0.5M 10% solution with TRIS 0.2M (pH 7.4) for 7 days. After decalcified period, they were dehydrated in crescent alcohol sequence and immersed in a mixture of alcohol 95% + resin (1:1) during four hours and in pure resin "overnight". In the next day, it was included in historesin (Leica Historesin). Each historesin block was transversally sectioned in long orientation of the teeth with semi-seriated slices (3µm) and submitted to histochemical reaction with TRAP (tartrate-resistant acid phosphatase).

## RESULTS

After two months, it was found in this work a little gingival recession and radicular exposure of teeth without function, inferior molars of the left side.

The stained histological glasses by tartrate-resistant acid phosphatase (TRAP) showed positive cells by red stain in cytoplasm (Fig 1B). These cells may be multinucleated placed at Howship lacunae or mononucleated (Figs. 2A, B). It was observed a great number of TRAP positive cells in the cervical and apical regions of the periodontal ligament, in the inferior first molars. These cells were not different between the sides of the control group and between the control and experimental groups on the right side, hyperfunction region, (Figs. 2C, D).

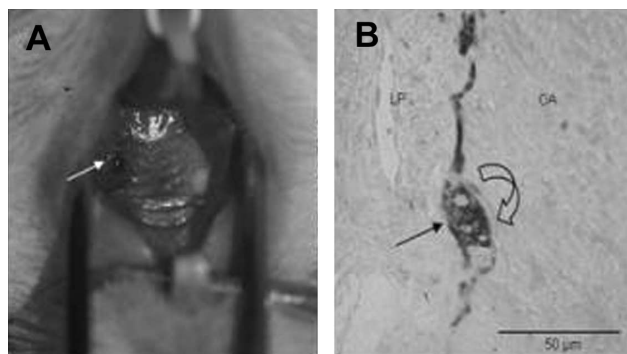


Fig. 1A. Occlusal view of gerbil showing the upper molars teeth extraction, on left side. Fig. 1B. Osteoclast observed by positive reaction using TRAP, red staining in cytoplasm (arrow), and the nucleus staining in green. It was possible to see the Howship lacunae (curve arrow), periodontal ligament (LP) and alveolar bone (OA). TRAP+Fast green 100X.

## DISCUSSION

The periodontal ligament tissue is thought to play an important part in the regeneration of the periodontium, but the precise function of the ligament tissue and cells is not yet clearly understood. Here we have attempted to clarify the histochemical alterations of the periodontal ligament in first molars, on the right side, after upper molars teeth extraction on the left side in gerbils.

After two months, it was found in this work a little gingival recession and radicular exposure of the teeth without function, inferior left molars, according to Burkland *et al.*, 1976 and Schropp *et al.*, 2003 works. This situation is explained by the fact that the opposite teeth extraction, permitted the continuous eruption of these teeth ahead of

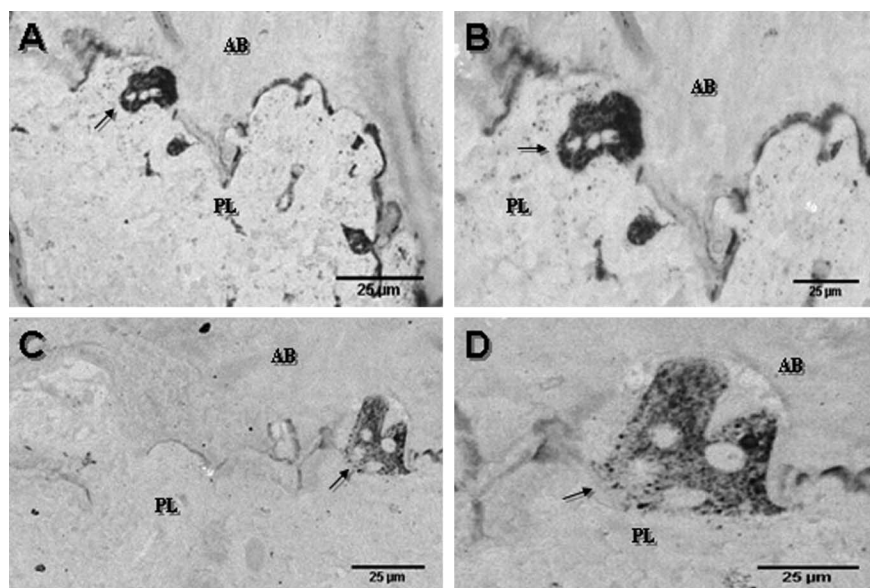


Fig. 2A. Panoramic view of the right first molar, control group, cervical region, showing TRAP positive cells (arrow), periodontal ligament, and alveolar bone (AB), TRAP+Fast green 20x.

Fig. 2B. Higher magnification of the TRAP positive cells on cervical region of the anterior figure, TRAP+Fast green 40x.

Fig. 2C. Panoramic view of the right first molar, experimental group, cervical region, showing TRAP positive cells (arrow), periodontal ligament, and alveolar bone (AB), TRAP+Fast green 20x.

Fig. 2D. Higher magnification of the TRAP positive cells on cervical region of the anterior figure, TRAP+Fast green 40x.

occlusal plane, probably by the cementary neoformation, although this process has been observed only in the apical region of the hypofunction side. These results, however, diverged to the data obtained in mice, after a long period of time in observation of antagonist teeth, it was not observed cement hyperplastic tissue or tooth supra-eruption, being that found intense resorption of remanescant alveolar bone and a little quantity of periodontal ligament in the interdental region, due to the lack of periodontal stimulation of the periodontal tissues by occlusal contact (Cohn), because the lack of function causes involution of the soft periodontal tissues, according to that was observed in humans and monkeys (Pihlstrom & Ramfjord, 1971).

The histological results of this study showed thick vessels in the periodontal ligament, principally in the apical region of the hemimandible submitted to hyperfunction activity, which is in agreement with Palcanis (1973) and Kvinnsland *et al.*, (1992) studies. They showed that an occlusal trauma can alter the vascular function and that blood pressure, even though the histological tissue presents a normal appearance. The numbers suggest an increase of the vessels in the periodontal ligament of the first molar, on the right hemimandible of the experimental group.

According to Cole & Walters (1987), tartrate-resistant acid phosphatase (TRAP) is able to show the osteoclasts presence. Thus, it has been used as a histochemical marker for osteoclasts in many studies (Brudvik & Rygh, 1993, 1994; Rody *et al.*, 2001; Kawarizadeh *et al.*, 2003, 2004; Kawasaki *et al.*, 2004; Fukushima *et al.*, 2005). It was observed in this study a high intensity staining for TRAP reaction in the hyperfunction hemimandible, which is explained by the intense bone remodeling process, represented by the increase of osteoclasts cells.

## CONCLUSION

It was possible to conclude that the TRAP reaction was able to show high intensity of osteoclastic activity in the hyperfunction hemimandible, explaining the functional changes in the periodontal ligament after teeth extraction.

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**RESUMEN:** El objetivo de este artículo es mostrar las alteraciones clínicas e histoquímicas del primer ligamento periodontal del lado derecho, después de la extracción del molar superior izquierdo en gerbiles (*Meriones unguiculatus*). Luego de dos meses, los ligamentos periodontales fueron retirados y procesados para el análisis histoquímico. Los resultados mostraron que la reacción de TRAP es capaz de evidenciar la actividad osteoclástica en la hiperfunción de la semimandíbula derecha, explicando los cambios funcionales del ligamento periodontal después de la extracción dental, siendo observada una pequeña recesión gingival y exposición radicular de los dientes sin función, en los molares inferiores izquierdos.

**PALABRAS CLAVE:** Ligamento periodontal; Histoquímica; maloclusión; Gerbil; *Meriones unguiculatus*.

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