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Microscopic evaluation of induced tooth movement in traumatized teeth: an experimental study in rats

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Abstract – The clinical management of orthodontic patients with dental trauma before or during the treatment is mainly founded on clinical experience, expert opinions, and individual case reports. It is proposed in the literature that teeth sustaining mild trauma with minor damage to the periodontium (e.g. subluxation) should be followed for a period of time before being subjected to orthodontic forces. A minimum period of 3 months has been proposed. In this study, we used an animal model to investigate whether shorter observation periods could be established in case of mild trauma. The periradicular region of rat molars was examined microscopically to determine the biological events of tooth movement started 15 and 30 days after intentional subluxation using an experimental method to induce dentoalveolar trauma. Thirty adult male Wistar rats were assigned to 6 groups ($n = 5$): Group 1 (control – no trauma/orthodontic movement); Group 2: the animals received an orthodontic device and were sacrificed after 7 days; Groups 3 and 4: dentoalveolar trauma (subluxation) was experimentally induced by the application of an axial force of 900 cN on the occlusal surface of the maxillary right first molar, and the animals were sacrificed after 22 and 37 days, respectively; and Groups 5 and 6: 15 and 30 days, respectively, after force application, an orthodontic device was installed and the rats were sacrificed 7 days later. In G5 and G6, the periodontal ligament and pulp tissue were rich in cellular elements and blood vessels, the alveolar bone was preserved, and the root surface presented only very small areas of surface resorption (cementum), maintaining the characteristics of normality. In conclusion, the microscopic alterations in the gingival and periodontal tissues in response to an experimentally induced mild dentoalveolar trauma simulating subluxation were not sufficient to contraindicate starting the orthodontic movement 15 and 30 days after trauma.

Dentoalveolar trauma is a subject of great interest in dental practice and has been considered an important public health problem not only because of its relatively high prevalence, but also because its functional and esthetic sequelae may have a substantial impact on the patient's life. High levels of interpersonal violence, traffic accidents, and a greater engagement of children in sports activities have contributed significantly to raise the prevalence of traumatic tooth injuries in the last years (1–4).

Most cases of dental trauma occur in patients aged 6–15 years, which is the age group that most often seek orthodontic care (2). Orthodontic movement of traumatized teeth or restart of orthodontic treatment interrupted owing to trauma has increased in daily clinical practice. However, the actual implications of dental trauma for patients requiring orthodontic treatment as well as the post-trauma waiting period have not yet been established experimentally, which hinders the establishment of treatment approaches elaborated on the basis of research data. A great part of the literature referring to dental trauma and orthodontic treatment comprises clinical trials with different methodologies, anecdotal case reports, retrospective review articles with small patient samples, and even empirical clinical experience (5–13). Orthodontists need scientifically based evidence that substantiates safe clinical protocols for orthodontic movement in traumatized teeth that can optimize the clinical time with minimal sequelae at the end of the treatment. When the orthodontic specialist is prepared when contemplating movement of traumatized teeth, whether by recognizing the trauma early and considering it in the treatment plan, or by diagnosing it when faced with an emergency, the physical, emotional, and esthetic impairments can be minimized and better outcomes are expected.

The International Association of Dental Traumatology's guidelines (14–16) for the management of traumatic dental injuries are useful for dentists and other health care professional for delivering the best care possible and achieving a more favorable prognosis. According to the guidelines for the management of luxations of permanent teeth (14), cases of mild trauma with minor damage to the periodontium (e.g. subluxation) do not require any major intervention, only stabilization of the tooth with a flexible splint for up to 2 weeks and follow up for 4–8 weeks.

A recent overview article on the influence of dental trauma on the management of orthodontic treatment (17) has stated that the recommended observation periods prior to commencing or restarting orthodontic tooth movement are dependent on the severity of the injury. The orthodontic forces can act as an additional trauma to the periodontium and thus caution and good sense are advised for planing each treatment. According to the authors (17), the current guidance for cases of subluxation is that the traumatized teeth should be followed for a period of 3 months before being subjected to orthodontic forces. However, the common sense in the literature is mainly founded on expert opinions and clinical experience. In this study, we used an animal model to investigate whether shorter observation periods could be established in case of mild trauma. The periradicular region of rat molars was examined microscopically to determine the biological events of tooth movement started 15 and 30 days after intentional subluxation using an experimental method to induce dentoalveolar trauma.

Material and methods

The research project was independently reviewed and approved by the Animal Research Ethics Committee of the Dental School of Araçatuba, São Paulo State University (UNESP, Brazil). All guidelines regarding the care of animal research subjects were strictly followed.

Thirty young adult male Wistar rats (*Rattus norvegicus albinus*) aged 3–4 months and weighing between 250 and 350 g were selected for the study. The animals were housed in plastic cages under climate-controlled conditions (12 h light/12 h dark; thermostatically regulated room temperature) and were fed a standard solid chow (RaçãoAtivadaProdutor; Anderson & Clayton S.A. Indústria e Comércio, São Paulo, SP, Brazil) and water *ad libitum*. All experimental procedures were performed under anesthesia. The animals received an intramuscular injection of ketamine hydrochloride (Vetaset®; Fort Dodge Animal Health, IA, USA; 0.07 ml per 100 g body weight) and xylazine hydrochloride (Dopaser®; Caleir S.A., Barcelona, Spain; 0.03 ml per 100 g body weight).

The animals were randomly assigned to six groups (n = 5): Group 1 (control – no trauma/orthodontic movement): the animals were followed up during the course of the study; Group 2: the animals received an orthodontic device and were sacrificed after 7 days; Groups 3 and 4: dentoalveolar trauma (subluxation)was experimentally induced by the application of an occlusogingival force of 900 cN on the occlusal surface of the maxillary right first molar using a tensiometer secured on a fully articulated support with adjustable steel shafts, and the animals were sacrificed after 22 and 37 days, respectively; and Groups 5 and 6: 15 and 30 days, respectively, after force application, an orthodontic device was installed and the rats were sacrificed 7 days later.

The dentoalveolar trauma was experimentally induced by the application of an occlusogingival acute force of 900 cN on the occlusal surface of the maxillary right first molar, using the methodology proposed by Pereira et al. (18) (Fig. 1).

In the animals of the groups subjected to induced tooth movement (G2, G5, and G6), a device similar to the model proposed by Heller and Nanda (19) (Fig. 2) was installed in the maxillary right first molar to promote mesialization by applying a controlled force of 50 cN, as checked with a precision tensiometer (Zeusan Exporting Ltda Campinas, São Paulo, Brazil).

After the experimental period established for each group, the animals were sacrificed by anesthetic overdose followed by decapitation. The right maxillas were removed, fixed in 10% buffered formalin for 24 h, and decalcified in acid 10% EDTA solution for 5 weeks. After decalcification, the specimens were embedded in paraffin and 6-μm-thick histological sections were serially cut from the mesial root of the maxillary right first molar (including the surrounding tissues). The cuts were made in a mesiodistal direction, along the long axis of the tooth, starting from the buccal surface toward the lingual surface. Eight to ten sections from the middle portion of the root were obtained for histological analysis, stained with hematoxylin and eosin, and observed under light microscopy.

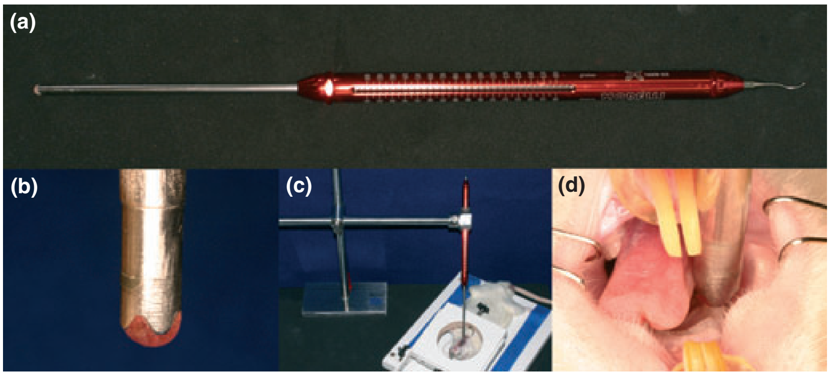

Fig. 1. Device used to promote experimental dentoalveolar trauma. (a) Tensiometer. (b) Active tip of the tensiometer adapted with acrylic resin. (c) Tensiometer adjusted to the rat molar. (d) End of the tensiometer rounded and fastened on the buccal alveolar crest of the maxillary right first molar.

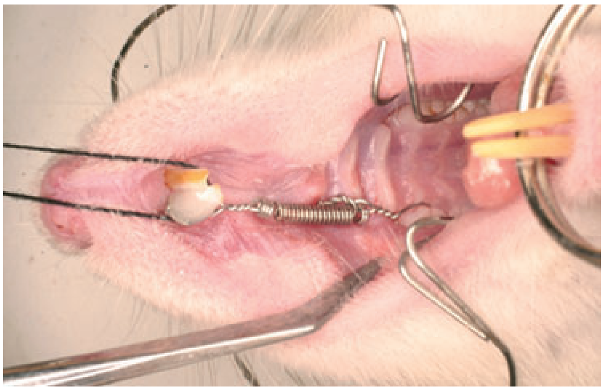

Fig. 2. Orthodontic device in position. Expanded coil exerting a force of 50 cN. Point where the splinting wire remainder is in contact with the buccal face of the maxillary right incisor with light-cured composite resin.

Results

The histological analysis comprised the coronal portion and the mesial root of the maxillary right first molar. Dentin, cementum, alveolar bone tissue, periodontal ligament (PDL), and the junctional epithelium of the gingival papilla were examined in these regions.

In Group 1, the PDL, dentin, cementum, alveolar bone tissue, and junctional epithelium of the gingival papilla were preserved, exhibiting normal characteristics. The junctional epithelium was juxtaposed to the cementum surface. The PDL was rich in collagen fibers and fibroblasts. Collagen fiber bundles were arranged horizontally in the cervical region, while in the middle and apical thirds, they were positioned in a more oblique arrangement. The collagen fibers presented an irregular arrangement in the periapical and furcal regions. Close to the apex, dentin was covered by primary cementum, which was covered by a layer of secondary cementum (Fig. 3). The alveolar walls were rich in osteoblasts and osteocytes.

In Group 2, only one animal presented some areas of resorption in the alveolar bone crest. Three specimens presented surface resorption (cementum) in the distal surface of the apical third of the mesial root. Osteoid tissue (fasciculate bone) was found in the alveolar bone crest (two specimens) and in the alveolar bone wall (five specimens). The major difference from the other groups was the presence of replacement resorption (ankylosis) in one specimen (Fig. 4).

In Group 3, three specimens presented some areas of resorption in the alveolar bone crest. Three specimens presented surface resorption (cementum) in the distal surface of the apical third of the mesial root (Fig. 5).

In Group 4, three specimens presented some areas of resorption in the alveolar bone crest. Two specimens presented surface resorption (cementum) in the distal surface of the apical third of the mesial root (Fig. 6). Osteoid tissue was found in the alveolar bone crest in one specimen and in the alveolar bone walls in two specimens.

In Group 5, two specimens presented some resorption areas in the alveolar bone crest. In one of these specimens, the most coronal portion of the alveolar bone crest was separated from the alveolar socket and exhibited clastic cells (Fig. 7). Surface resorption (cementum) was observed in three specimens in the distal surface of the apical third of the mesial root. Newly formed bone tissue (fasciculate bone) was found in the alveolar bone crest (two specimens) and in the alveolar bone wall (three specimens).

In Group 6, only one specimen presented resorption areas in the alveolar bone crest. In this specimen, the most coronal portion of the crest was separated from the alveolar socket. In two specimens, surface resorption (cementum) was observed in the distal surface of the apical third of the mesial root, and one of these specimens also exhibited surface resorption in the distal

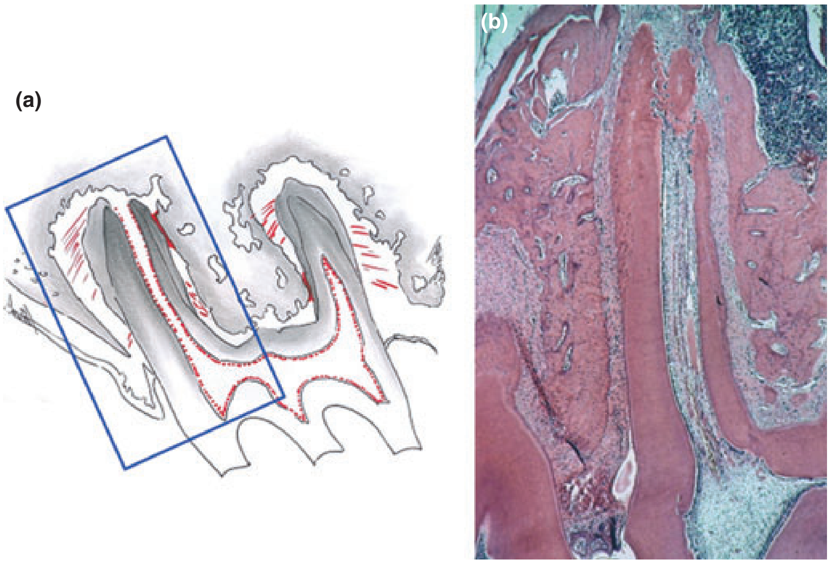

Fig. 3. Group 1. (a) Schematic presentation of a cross section of a molar tooth with the examined area delineated by a box. (b) Corresponding photomicrograph of the boxed region shown in a. Mesial root of the maxillary right first molar with primary cementum covering the dentin of the apical region. HE. 25× magnification.

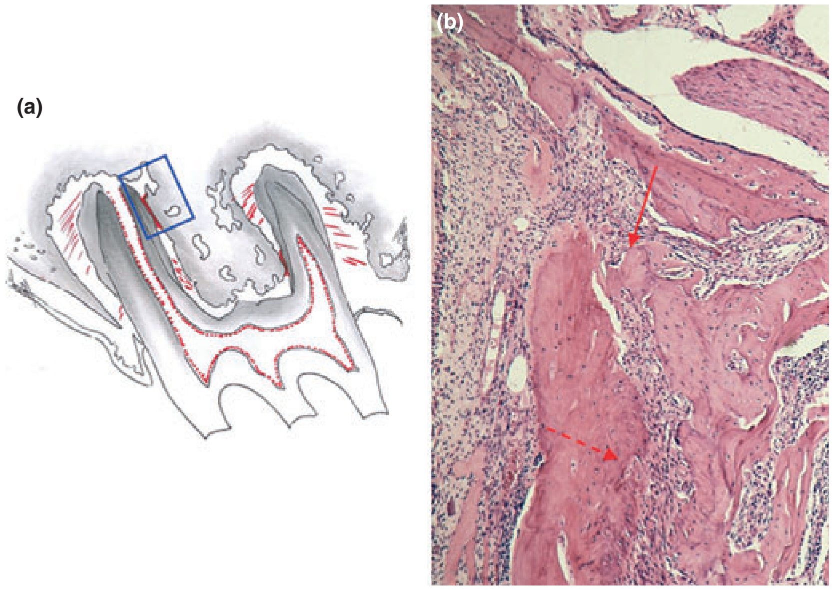

Fig. 4. Group 2. (a) Schematic presentation of a cross section of a molar tooth with the examined area delineated by a box. (b) Corresponding photomicrograph of the boxed region shown in a. Apical third of the distal surface with areas of ankylosis (arrow) and surface resorption – cementum (dotted arrow). HE. 63× magnification.

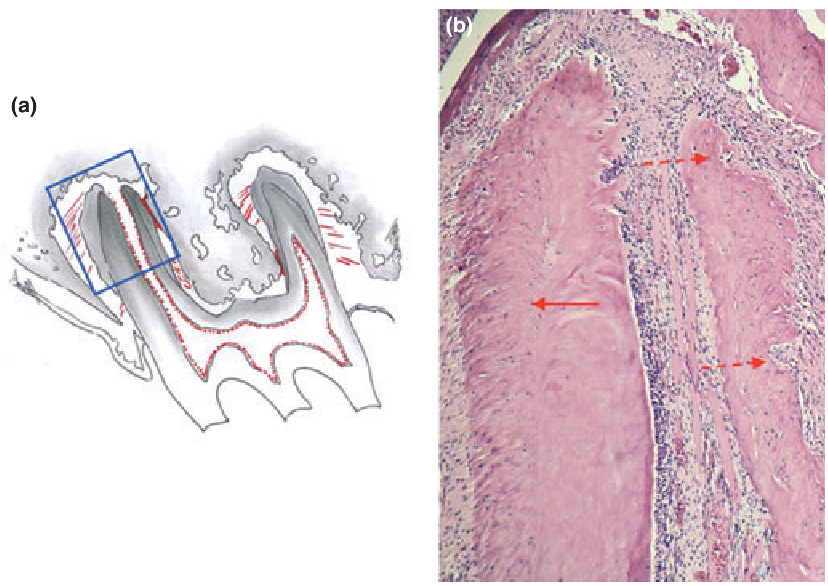

Fig. 5. Group 3. (a) Schematic presentation of a cross section of a molar tooth with the examined area delineated by a box. (b) Corresponding photomicrograph of the boxed region shown in a. Apical third of the mesial surface covered by a thicker layer of cementum (arrow) and surface distal with areas of surface resorption – cementum (dotted arrows). HE. 63× magnification.

surface of the apical third of the mesial root. Osteoid tissue was found in the alveolar bone crest in four specimens and in the alveolar bone wall in two specimens. In three specimens, a hyaline tissue was observed originating from the region of the contact of the distal surface of the root with the alveolar bone wall (Fig. 8).

Discussion

In the present study, the experimental dentoalveolar trauma induced by the application of an occlusogingival (axial) acute force on the occlusal surface of the maxillary right first molar of rats produced clinical and histological alterations that are compatible with those caused by frontal impacts, such as observed in concussion and subluxation (14, 20). Animal models using rats have been proposed for the study of orthodontic movement (21).

Clinically, the teeth subjected to dental trauma and/or orthodontic movement presented mobility without dislodgment from the socket after the induction of trauma. At the moment of sacrifice, however, mobility was no longer present. The animals subjected only to the induction of dentoalveolar trauma and sacrificed after 22 (G3) and 37 (G4) days presented mild microscopic alterations in the gingival and periodontal structures. Most microscopic alterations were concentrated in the alveolar bone crest and in the distal surface of the apical third of the mesial root. In both groups, the alveolar bone crest exhibited resorbed areas. In the distal surface of the apical third of the mesial root, small areas of surface resorption (cementum) were

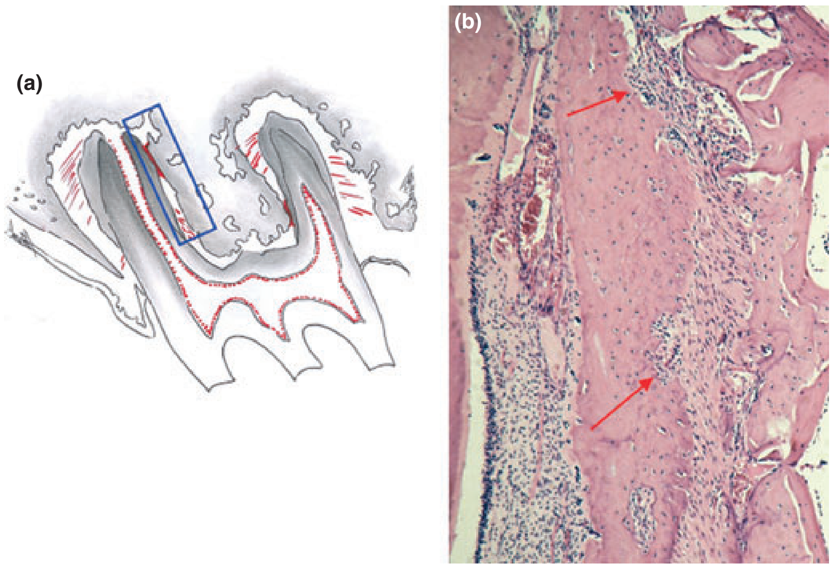

Fig. 6. Group 4. (a) Schematic presentation of a cross section of a molar tooth with the examined area delineated by a box. (b) Corresponding photomicrograph of the boxed region shown in a. Apical and middle thirds of the distal surface with areas of surface resorption – cementum (arrows). HE. 63× magnification.

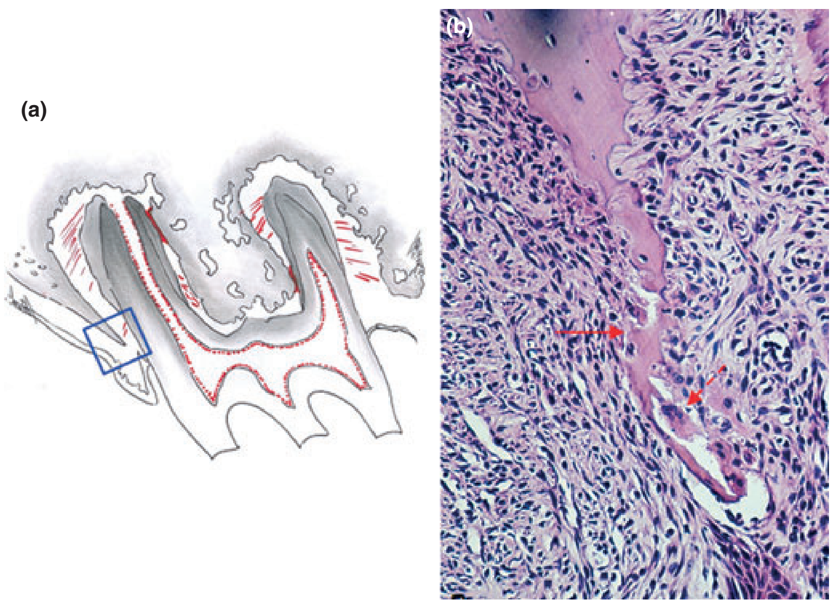

Fig. 7. Group 5. (a) Schematic presentation of a cross section of a molar tooth with the examined area delineated by a box. (b) Corresponding photomicrograph of the boxed region shown in a. Alveolar bone crest fragmentation of the coronal portion (arrow) with the presence of clasts (dotted arrow). HE. 160× magnification.

found in most specimens of these groups. However, the major difference between these groups was the presence of newly formed bone tissue (osteoid tissue) in the entire extension of the alveolar bone crest and the alveolar bone wall, exhibiting fasciculate bone-like aspect. The presence of osteoid tissue (fasciculate bone) suggests repair of the area, as it is frequently present under conditions of normality in the periodontium of murine molars.

Although surface resorption (cementum) was the most significant alteration observed in G3 and G4, an important finding was that these areas presented new collagen fiber reattachment and were recovered by new cementoblasts.

All types of root resorption (surface resorption, inflammatory resorption, and replacement resorption – ankylosis) start with a damage to the cementum layer that protects the root surface (22). In traumatized teeth with mild damage to the cementum, repair takes place by repopulation of the damaged surface by cementoblasts. The same type of repair is observed in the resorption occurred in response to orthodontic movement. On the other hand, Kindelan et al. (17) have reported that cases of more severe damage to the cementum result in replacement resorption (ankylosis) by osteoblast infiltration in the damaged area. According to those authors, during the post-trauma repair period, additional damage to the cementum will accentuate the inflammatory stimuli, prolonging the destructive phase and increasing the risk of resorption and ankylosis. Before starting the orthodontic treatment, a repair period should be allowed for PDL repair (17).

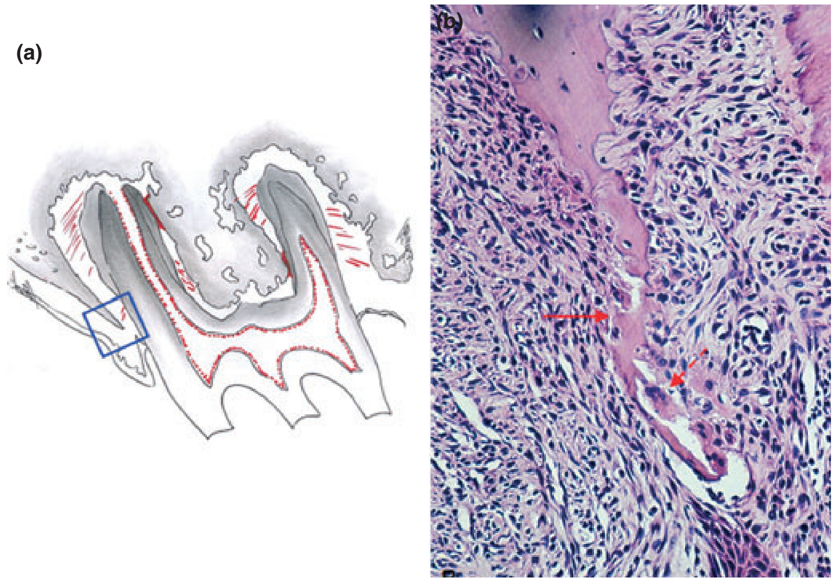

Fig. 8. Group 6. (a) Schematic presentation of a cross section of a molar tooth with the examined area delineated by a box. (b) Corresponding photomicrograph of the boxed region shown in a. Apical third of the distal surface with the presence of hyalinization area (arrow). HE. 160× magnification.

Andreasen and Kristerson (23) have found that mild damage to the cementum layer (1–4 mm^2) leads to transient ankylosis, which disappears by the 8th week post-trauma; in more severe damage (9–16 mm^2), ankylosis persisted for more than 8 weeks.

According to Flores et al. (14), teeth that suffer mild trauma (e.g. subluxation) must be followed clinically and radiographically in the first 4–8 weeks within the 1st–5th year post-trauma. The treatment consists of stabilizing the traumatized teeth with a flexible splint for up to 2 weeks to provide more comfort to the patient. The authors also reinforce the importance of the pulp vitality test. The teeth may not respond initially to stimuli, indicating a transient pulpal alteration, and monitoring must be performed to determine the definitive diagnosis of the pulpal condition.

The results of this study, which had a short observation period prior to the start of the orthodontic movement, do not agree with those of other studies that indicate longer observation periods for cases of subluxation (6, 17). Normal histological characteristics were observed in the periradicular region of teeth subjected to trauma only (G3 and G4), which indicate that orthodontic forces could be applied. In the animals subjected to tooth movement 15 and 30 days after experimentally induced dentoalveolar trauma (G5 and G6), the PDL and pulp tissue were rich in cellular elements and blood vessels, the alveolar bone was preserved, and the root surface presented only very small areas of surface resorption, maintaining the characteristics of normality.

According to Kindelan et al. (17), the observation period that should precede orthodontic movement after dental trauma depends on the severity of trauma, and this period is necessary to allow for PDL healing. According to those authors, after traumatic injuries causing mild periodontal damage (concussion and subluxation) before or during the treatment, the waiting period for orthodontic treatment should be of 3 months. However, the authors comment that there are few studies addressing the influence of root resorption in traumatized teeth during orthodontic treatment, and their results are not consensual. Owing to the small number of patients that sustain dentoalveolar trauma during the orthodontic treatment, there are no published prospective or retrospective studies to guide about the best management under these conditions, and thus the waiting period is based on clinical experience, expert opinions, and individual clinical cases. The authors also reported that the patients that sustain dentoalveolar trauma previously to the orthodontic treatment must be warned about not only the increased risk of root resorption owing to the action of orthodontic forces, but also the increase in the risk of future trauma episodes during orthodontic treatment, which might have an impact on the prognosis of tooth movement and on treatment duration.

In the present study, the microscopic findings in the experimental groups were similar to those observed in the control group, which simulate normal conditions. This result suggests that a shorter post-trauma waiting period appeared to be sufficient to allow for periodontal healing. It may be inferred that the period elapsed from the moment of trauma to the induction of tooth movement might have contributed to the repair, leading to a condition of 'normality' that frequently occurs in a short observation period. In this way, the waiting period is favorable as far as the local pain and general post-trauma recovery are concerned; however, regarding the repair of periodontal tissues and structures, such a waiting period seems not be necessary in case of mild trauma, such as subluxation. Mild dentoalveolar trauma (subluxation) did not promote significant microscopic alterations in the gingival and periodontal tissues that could contraindicate orthodontic tooth movement, as the integrity of the PDL, pulp, root and alveolar bone was preserved.

Conclusion

The periradicular region of rat molars subjected to orthodontic movement 15 and 30 days after an experimentally induced mild dentoalveolar trauma simulating subluxation was similar to those of the control group and the group subjected to dentoalveolar alone, maintaining the conditions of normality. Further research should be performed with shorter observation periods to determine the periodontal repair period in cases of subluxation.

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