**Original Research Article**

**EFFECTS OF FORCES USED IN TOOTH MOVEMENT ON THE PERIODONTIUM OF TEETH WITH EXTRUSIVE LUXATION - EXPERIMENTAL STUDY IN RATS**

**ABSTRACT**

**Aims:** This study assessed the effect of different types of orthodontic forces (continuous (C), continuous interrupted (Ci) and intermittent (I)) on the amount of tooth movement (TM) and root resorption (RR) mesio-vestibular and intermediate (RRmv, RRi, respectively) of teeth submitted (L) or not (nL) to extrusive luxation (EL). Data on hyaline areas were also assessed.

**Study design:** Experimental research

**Methodology:** Forty-eight Wistar rats were randomly divided into 8 groups (n=6) according to the combination of independent variables (the type of force and luxation): the moved [nL-C, nL-Ci, nL-I, L-C, L-Ci, L-I] and the non-moving [L-nM and nL-nM (control)]. The EL of the first right upper molar was performed under a force of 1500 cN for 10 seconds. After 15 days, TM was initiated with a force of 50 cN, with the three types of force (C, Ci, I). On the 14th day, the amount of TM was measured, and the animals were euthanized, the parts processed, included, cut at the cervical level of the molars, and submitted to staining with hematoxylin and eosin (HE).

**Results:** The results showed more significant movement for the C and Ci forces when compared to I in the groups submitted to luxation (p<0.05). The luxation factor did not influence tooth movement, except for the Ci force (nL-Ci =0.25 x L-Ci=0.44) (p<0.05).

**Conclusion:** The amount of RR was not influenced by the type of force nor by the EL. Regarding the number of hyaline areas, it was not possible to visualize differences between the groups, under the conditions of this study.

**Keywords:** Dental Trauma, Dental Movement, Root Resorption.

**1 – INTRODUCTION**

Orthodontics is very concerned with dentoalveolar trauma (DT), especially those involving periodontal support since the success of orthodontic therapy depends on the integrity of these structures [1, 2]. Some studies have shown that the prevalence of DT is high in the population, especially among children and adolescents [3, 4], and that orthodontic interventions are common in these age groups. About ten percent of patients seeking treatment with orthodontists have suffered some dentoalveolar trauma [5]. This shows the importance of behaviors to be adopted by the professionals concerning possible complications during orthodontic treatment in these patients, as well as the concern about ]the ideal time to start treatment in patients who have suffered DT [6].

Andreasen and Andreasen classified the types of DT, which involve periodontal tissues, according to their intensity in concussion, subluxation, extrusive, lateral and intrusive luxations, and avulsion. Among these, extrusive luxation (EL) is defined as a partial displacement of the tooth out of its alveolus [7], thus characterized by elongated appearance, mobility, and negative response of the tooth to the sensitivity test. Radiographs show an increase in periodontal ligament space [8]. Despite being one of the most common traumas, data on induced tooth movement (ITM) in teeth with this type of luxation are still based on case reports and the opinion of authors/clinicians.

In orthodontics, two different types of forces are employed: continuous and intermittent; the duration of the initial magnitude is different between the two. Consequently, results in tooth movement and negative impacts on the tooth and adjacent tissues may differ according to the force employed during orthodontic treatment [9]. Clinically, continuous force is performed by high-elasticity wires and springs (NiTi alloy); on the other hand, when wires or springs with reduced elasticity and shape memory (steel) are used, the magnitude of force is gradually reduced and reaches a level unable to allow the continuity of tooth movement, and is then classified as interrupted continuous force [9]. The intermittent force acts for a short time and is eliminated by removing the force-generating device. This condition is observed with the use of removable, extraoral, and elastic appliances [10].

Root resorption (RR) is one of the main sequel seen in DT, ITM, and, especially, when there is an association between trauma and orthodontic movement. The RR occurs through the recruitment of clastic cells that resorb mineralized tissues, which can lead to tooth loss in severe cases [2, 11-13]. According to Tondelli, in teeth without a history of TD, induced tooth movement (ITM) with continuous forces would result in more significant root resorption [14].

Based on the above, it was considered appropriate to assess what type of force would be most appropriate for ITM in teeth with a history of EL. Therefore, this study aimed to assess the effect of different types of orthodontic forces (C, Ci, I) on the amount of TM and the periodontium of rats submitted to LE.

2 - METHODOLOGY

# 2.1 - Sample selection and distribution

The experimental procedures adopted in this study were approved by the Ethics Committee for Animal Experimentation of the Western Paraná State University (UNIOESTE), Cascavel, Paraná, Brazil (Appendix 1). All procedures were performed in May and June 2018.

A sample of 48 rats (n=6) was calculated considering the variable root resorption area (Kruskal-Wallis), with a significance level of 5% and test power of 80% (GPower 3.1 software, University of Düsseldorf; Fracalossi, 2009; Nakano et al., 2014).

Forty-eight male rats (Rattus norvegicus albinusWistar, approximately 90 days) were used, weighing between 250 and 300 g at the beginning of the procedures, originating from the UNIOESTE bioterium. The animals were kept in collective polyethylene cages under controlled conditions, the light cycle of 12/12 hours, and constant temperature (22°C ±2°C), fed with solid food and water *ad libitum*. Before the experimental procedures, they were conditioned for 7 days for adaptation. The cages were sanitized on alternate days.

The animals were anesthetized both for trauma application and for installation and activation of the ITM spring, via intraperitoneal with ketamine hydrochloride (Dopalen, Sespo Ind. e Com. Ltda., Jacareí, SP, Brazil; 75 mg/Kg of weight) and xylazine hydrochloride (Anasedan, Agribrands do Brasil Ltda., Paulínia, SP, Brazil; 15 mg/kg of weight).

The animals were randomly divided into 8 groups (n=6), according to the combination of independent variables[(the type of force/no movement (sM) and presence or absence of LE): nL-C, nL-Ci, nL-I, L-C, L-Ci, L-I, L-nM and nL-nM (control group).

# 2.2 - Application of extrusive luxation

The EL was experimentally induced in the first right upper molar in 24 animals by a single trained and calibrated operator. The rats were placed on a stretcher in the dorsal decubitus position. The legs were trapped to restrict movement, the head still, and the mouth remained open during the procedures. Then, the trauma followed the methodology described by Costa *et al.* [15]

# 2.3 - Induced tooth movement (ITM)

Fifteen days after the EL, the ITM was started using a device according to the Heller & Nanda method (1979) [16] modified by replacing the steel spring with nickel-titanium and inserting a light-curing resin (Z100, 3M, St. Paul, MN, USA) in the cervical region of the incisor to improve wire retention. This procedure enabled the activation and deactivation of the spring, as well as the protection of the animal's mucosa. Subsequently, the springs were installed in such a way as to result in one of the different types of orthodontic forces, following the methodology used by Tondelli. [14] (Figure 1)

 

Figure 1 - Demonstration of groups, periods of activation, deactivation or removal of springs, and the day of the animals' euthanasia. (Source: Adapted from Tondelli [14])

During the experiment, after installation of the appliance, the feed supplied to the animals was moistened and ground to reduce the possibility of breaking it [14]. One animal was lost in the I-L group (n=5).

# 2.4 - Euthanasia and hytotechnical processing

On the 14th day of the experiment, the animals were euthanized by anesthetic overdose and then decapitated. The maxilla was preserved from the incisors to the third molars. The maxilla was fixed in 10% formaldehyde for 48 hours, washed in running water for 24 hours, and decalcified with Allkimia® (Allkimia, Campinas, São Paulo, Brazil). Afterward, the pieces were dehydrated for 3 hours in 80, 90, and 100% alcohol, clarified in xylol 100% for 40 minutes and included in paraffin blocks [17]. The sections were cross-sectioned 5µm thick, from the dental crown to the apex, followed by conventional staining with hematoxylin and eosin (HE).

# 2.5 - Quantitative analysis of tooth movement

#  The amount of tooth movement was obtained by the difference between the distances from the mesial surface of the maxillary first molar to the distal of the maxillary third molar on the left (not moved) and right [18, 19] sides. They were measured with a digital pachymeter (Mitutoyo, São Paulo, Brazil) by a calibrated evaluator. The right and left sides (moved and non-moving, respectively) were compared, and the induced movement in the right molar was measured.

# 2.6 - Scanning of histological sections

# For histological analysis, an optical microscope (Olympus BX61) was used, to which an Olympus DP71 digital camera (Olympus Corporation, Japan) was attached to obtain the photomicrographs, with increases of 40x, 100x and 200x. The images were analyzed with the help of Image Pro Plus software (Media Cybernetics, USA). The region of interest was the cervical region of the periodontium below the root separation area. This region was chosen because it allowed full visualization of the mesial-vestibular and intermediate roots of the right maxillary first molar. The slides were identified and coded in order to establish the blinding of the examiner [14, 15].

**2.7 - Analysis of the amount of root resorption**

With the help of Image Pro Plus software, the photomicrographs of the mesio-vestibular and intermediate roots were analyzed. At first, the total area of each root portion was determined. Subsequently, the presence or absence of root resorption was verified. Microscopically, this biological phenomenon is identified by the presence of Howship gaps in the cemental surface, which may reach the dentin layer, with or without the presence of clastic cells inside. The area of the root surface at the site presents the loss of the cementoblastic layer and direct exposure of the cementoblastic and dentinal tissue to the resorptive action of the clastic cells[20]. When present, the RR region was contoured and measured. Thus, the areas of resorption were quantified, and the values were expressed as percentages of these roots total areas. Photomicrographs were selected with an increase of 100x (3). (Figure 2)

 

 Figure 2 - Outline root resorption areas on the intermediate root (HE. 100X)

**2.8 - Analysis of the quantity of the hyaline area**

Photomicrographs were used to determine whether or not hyaline areas occurred in the periodontal ligament of the analyzed roots. The extracellular matrix of the periodontal ligament alters its biochemical and organizational relationship of its components, due to hypoxia generated in the tooth after the application of force. This results in microscopically poor cell areas with homogeneous eosinophilic appearance (frosted, ground-glass type), called hyaline areas of the extracellular matrix [20]. When present, these areas were circumvented and quantified as a percentage of the respective areas concerning the total area of the periodontal ligament of their roots. Photomicrographs were selected with an increase of 100X [3]. (Figure 3)

 

Figure 3 - Hyaline areas delimited in the periodontal space in the mesial vestibular (HE. 100x)

# 2.9 - Statistical analysis

The statistical analyses were made in the BioEstat 5.3 program (Instituto Mamirauá, Belém, Pará, Brazil). The data were submitted to the Shapiro-Wilk normality test, and the groups were compared by analysis of variance (ANOVA) and Tukey's post-test (0.05) when the criteria of normal distribution and homoscedasticity were met. If the data did not show normal distribution, the non-parametric Kruskall-Wallis test was used, with Dunn's post-test (when necessary), with a significance level of 5%.

# 2.10 - Method error

Inter-examiner and intra-examiner concordance for measurements of RR area and hyaline areas were assessed by the intraclass correlation test and demonstrated, respectively, high reproducibility (0.892) and reliability (0.923).

**3 – RESULTS**

**3.1 – Amount of Dental Movement**

Table 1 shows the significant statistical difference between the groups regarding the amount of movement. The forces C and Ci presented more significant movement when compared to force I in the groups submitted to LE (*P*<0.05). The luxation factor promoted a difference in this variable, when the interrupted continuous force was applied (nL-Ci=0.25 x L-Ci= 0.44) (*P*<0.05).

|  |
| --- |
| **Groups** |
| nL-C | nL-Ci | nL-I | L-C | L-Ci | L-I |
| 0.295(0.083)AB | 0.2533(0.1084) A | 0.2033(0.0728) A | 0.3733(0.1786) B | 0.4417(0.1886)B | 0.2167(0.0677)A |

**Table 1** - Amount of tooth movement (mm). Average (standard deviation), n= 6. Different letters indicate the statistical difference between groups. Equal letters indicate statistical similarity between groups. (ANOVA, Tukey's post-test, *P*<0.05)

**3.2 – Root resorption**

The data related to the percentage assessment of root resorption about the total perimeter of the MV, and I roots are described in Table 2.

Regarding root resorption, there was a statistically significant difference between the groups for root MV (*P*=0.00200) and root I (*P*=0.0016). The control group (nL-sM) was different from all other groups.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  **Root** |  |  |  | **Groups** |  |  |  |  |
|  | nL-C | nL-Ci | nL-I  | nL-sM | L-C | L-Ci | L-I |  L-sM |
| **mesium****VESTIBULAR (MV)** | 0.441(0.351)A | 1.1877(1.0327)A | 1.5056(1.5075)A | 0.1028(0.2310)B | 0.4440(0.4208)A | 0.6862(0.6979)A | 1.5862(1.6916)A | 0.4376(0.4296)A |
| **INTERMEDIate****(I)** | 0.5090(0.4620)A | 0.9027(0.7401)A | 0.7464(0.6826)A | 0.0000(0.0000)B | 0.4056(0.5513)A | 1.1467(0.9669)A | 1.0502(0.8471)A | 0.5844(0.7617)A |

**Table 2** – Root resorption area, in µm2, expressed in % (average and standard deviation). In the lines, equal letters indicate statistical similarity between the groups. Different letters indicate a statistically significant difference between groups. (Kruskal-Wallis post-test Dunn, p<0.05).

**3.3 – Hyaline area**

The data related to the percentage assessment of hyaline areas regarding the periodontal ligament total area of the two roots analyzed are presented in Table 3.

No statistically significant differences were found between the groups when comparing the amount of hyaline areas in the mesiovestibular and intermediate roots (*P*=0.76 and *P*=0.67, respectively).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **root** |  |  |  | **groups** |  |  |  |  |
|  | nL-C | nL-Ci | nL-I | nL-sM | L-C | L-Ci | L-I | L-Sm |
| **mv**  | 1.176(3.638) | 1.1708(4.2314) | 1.2798(2.1285) | 0.0000(0.0000) | 0.9368(1.7804) | 0.5856(9.8897) | 0.6574(1.7106) | 0.5939(1.6646) |
| **I** | 0.185(0.584) | 1.4752(3.2600) | 0.9095(2.1598) | 0.0000(0.0000) | 0.0000(0.0000) | 9.9342(13.6261) | 0.0000(0.0000) | 0.0000(0.0000) |

**Table 3** – Percentage values of hyaline areas in the periodontal ligament of the two roots, in µm2, expressed in % (average and standard deviation). Kruskal-Wallis *P*<0.05.

**4 – DISCUSSION**

In this study, a device was used to perform the trauma that enabled the standardization of the magnitude and angle of force application, being these positive and differential characteristics of this method, which generated similar responses throughout the sample [15]. This method of extrusive luxation resulted in a displacement of the dental element in the occlusal-mesial direction, allowing one to assess the effects of the association between the extrusive luxation and the subsequent induced tooth movement.

The magnitude of force applied in experiments for rat molar ITM ranges in the literature from 0.8cN[21] to 100cN[22]. In this study, a force magnitude of 50cN was employed using a NiTi closed spring, within the range recommended by the specialized literature. [15, 23].

The selection of the cross-section in the cervical region of the roots was based on the concomitant visualization of the roots of interest, the observation of the entire root perimeter, and the quantity and quality of the cementum. Thus, it was possible to have a notion of the tooth movement behavior in traumatized or non-traumatized teeth, with the different types of force in the period studied.

The quantitative analysis of tooth movement in this study followed the method described in the literature by other authors [18, 19]. When analyzing the movement in the groups that did not have traumatized teeth, it was observed that there was no difference between them. This result corroborates the findings of Hayashi and his collaborators [24] in establishing that the magnitude and duration of force are essential factors in stimulating the recruitment of osteoclasts in the periodontal ligament and that the amount of initial tooth movement is similar for both continuous and intermittent or interrupted forces.

Considering the presence of extrusive luxation in the moved tooth, groups L-C and L-Ci were similar but statistically different from the L-I group. The same behavior was established by Tondelli's study in 2011 [14], assessing at different times the effect of the same ITM device.

The process of root resorption begins after the death of the cementoblast layer that covers the root, caused by excessive cellular stress in the periodontal ligament, allowing access of clastic cells to the dental root mineralized surface [10, 25]. In this study, when analyzing root resorption in the mesiovestibular and intermediate roots, there was a statistically significant difference between the groups for both roots. The group without trauma - without movement was different from all other groups [14]. The same author, when studying the forces in non-traumatized teeth, also found no difference in root resorption data. In contrast, Costa [15] was only able to quantify root resorption differently when he induced tooth movement only 3 days after trauma.

The induced tooth movement time and consequent reapplications of orthodontic force used in this research may not have been sufficient to generate more defined root resorption, which would be observed in humans with six months of tooth movement [10, 26]. Another notable fact is that there was a high standard deviation for root resorption percentages, which leads us to suppose that this phenomenon is individual, that is, each organism, or more specifically each traumatized tooth, would have a specific response in terms of the amount of root loss by resorption. This leads to the need for orthodontists to be aware of this sequelae and to monitor the patient radiographically every six months.

It should be noted that these observations were obtained in the cervical regions analyzed through cross-sectional sections. We did not assess the medial and apical regions of the roots, where resorption phenomena also occur. The choice of this region was based on the concomitant visualization of the roots of interest, on the observation of the entire root perimeter, following the methodology chosen by Cuoghi, who also emphasized the process of root resorption [26]. Similar results were exposed by Zamalloa in 2009, who, similarly to this study, also found no difference between continuous and interrupted force when using the same magnitude of force for 8 days [27].

The hyaline area is described as a homogeneous region free of nuclei or cells in the periodontal ligament [28]. These cells disappear by migrating or necrotizing, leaving the site only with the modified extracellular matrix that assumes a vitreous aspect [10].

In the microscopic analysis of the periodontal ligament, some hyaline areas could be histometrically quantified in the mesio-vestibular and intermediate roots. However, under the conditions of this study, it was not possible to identify differences in hyaline areas in the analyzed roots when comparing the three types of force, associated or not with the TD.

Based on the Tengku study, the force used in this study (50cN) is considered heavy, since it exceeds the blood capillary pressure of the rat's dental structure [29]. Other authors, such as Consolaro and Fracalossi, stated that 75cN of force magnitude could be considered moderate for the mesial-vestibular root and high for the smaller roots as the intermediate. This information may justify the low occurrence of hyalinization in this research.

Our result regarding the intermediate root agrees with that found by Tondelli [14] for that same root portion, using the same magnitude of the force, when comparing the same three types of forces. In this research, as in Costa's, hyaline areas were observed in a non-significant manner, even with the application of a force considered heavy [15]. These results suggest that the induced tooth movement time, as well as the activation regime (only two activations), may not have been sufficient to generate a higher concentration of hyalinization areas.

Considering orthodontic therapy, in which the doctor's office serves patients who have already suffered some dental trauma, including extrusive luxation, the objective would be to correct malocclusion without resulting in damage to the teeth and adjacent tissues. From the results of this study, it can be inferred that the force CI was the one with the highest rate of tooth movement. Clinically, this would mean a shorter treatment time with the use of force CI, and this type of force also showed small damage to the root of the traumatized and moved tooth. Future methodologies, with different times and materials, will be valid to observe better the behavior of teeth that have suffered some trauma in the face of orthodontic mechanics.

5 - CONCLUSIONS

 Under the conditions of this study, it can be concluded:

 - The amount of tooth movement in teeth submitted to extrusive luxation was influenced by the type of force, which was more significant for the continuous and continuous interrupted forces.

 - The amount of root resorption was not influenced by the type of force or by the extrusive luxation.

- The induced experimental conditions (trauma and ITM) were not sufficient to generate alterations in the hyaline areas.

**CONSENT**

It is not applicable.

**ETHICAL APPROVAL**

This study was in accordance with the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and were approved by the Committee on Ethics in the Use of Animals (CEUA) of UNIOESTE.

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mesial vestibular root. (HE 100x)