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EFFECT OF LOCAL ADMINISTRATION OF HYALURONIC ACID ON ORTHODONTIC TOOTH MOVEMENT IN A RABBIT MODEL

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ABSTRACT

INTRODUCTION: Using a biocompatible material to control tooth movement and enhance anchorage may be a useful method to help the orthodontist to give better treatment results.
OBJECTIVES: To Assess the effect of hyaluronic acid (HA) local injection on orthodontic tooth movement.
MATERIALS AND METHODS: The sample size was 15 male New Zealand rabbits. Randomized prospective split mouth trial was conducted on each rabbit. A mesializing force was applied in both sides for 21 days. **Group A (control)**: Saline was injected on day 0. **Group B:** HA 1% was injected. Tooth movements were measured on 3D models. Histological analysis of alveolar bone modeling was done in the end of this experiment.
RESULTS: The quadrant receiving HA showed less amount of tooth movement than that in the control quadrant. Local administration of HA yielded 10% less tooth movement. Histological analysis revealed less resorptive activity in the quadrant receiving HA.
CONCLUSION: There was statistically significant reduction in orthodontic tooth movement magnitude after local HA administration as well as reduction in bone resorptive activity.
KEYWORDS: Hyaluronic acid, Rabbits, Tooth movement.

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INTRODUCTION

Successful orthodontic treatment necessitates a full control over tooth movement. If the orthodontist failed to prevent any unwanted tooth movement this will lead to unsatisfactory results for both the patient and the clinician. Anchorage loss during orthodontic treatment and relapse after finishing are the most common unwanted tooth movements (1).

Our ability to control completely over tooth movement in all aspects is limited. In addition, clinicians, to address these limitations, often implement bulky acrylic or extraoral devices that are often inefficient specially when combined with the ever-challenging issue of uncooperative patients (2). Research in the area of orthodontic tooth movement has grown rapidly and helped to expand the current knowledge about the biological basis of orthodontics (3).

Various pharmacological agents, whether administered systemically or locally, have been tested to hinder orthodontic tooth movement in several animal models, including nonsteroidal anti-inflammatory drugs, (4, 5) simvastatin (6) and bisphosphonates (4). Each pharmacological agent has its mechanism of action through which modification of supporting tissues remodeling process took place leading to impediment of

orthodontic tooth movement. Based on these findings, pharmacological therapeutic strategies may be a better choice to control over alveolar bone remodeling and impede tooth movement.

Hyaluronic acid (HA), is a glycosaminoglycan with a high molecular weight, known also as hyaluronan, formed of repeated non-sulfated disaccharide units of N-acetyl glucosamine and D-glucuronic acid (7, 8). In addition to controlling fibroblast proliferation, HA has been shown to have an important function in cellular signaling, morphogenesis, and matrix organization (9-13). Moreover, hyaluronan shows also bacteriostatic (14, 15), anti-inflammatory (16), osteoinductive (17-20), and pro-angiogenic properties (21). Owing to its hygroscopic nature, HA has various functions, it affects the physical properties and the hydration of the extracellular matrix significantly. It can also interact with various receptors resulting in the activation of signaling cascades which influence cell migration, proliferation, and gene expression (22).

Sasaki and Watanabe (20) found that high-molecular HA may be able to accelerate new bone formation through differentiation of mesenchymal cell in bone wounds. Kim et al (23), showed that owing to the bacteriostatic, anti-inflammatory and osteoinductive properties, HA may enhance bone formation and help in wound healing acceleration. Sadikoglu et al (24), conducted an animal study using rat model to evaluate the effect of different molecular weights of HA on bone formation after interpremaxillary suture expansion. They concluded that local injection of high molecular weight HA in the interpremaxillary suture of rats after rapid maxillary expansion lead to stimulation of new bone formation, which can be effective to decrease the retention phase of treatment and also can minimize the risk of relapse.

The hypothesis of this study was that HA's osteoinductive effects and its anti-inflammatory properties in

addition to its ability to decrease alveolar bone resorption might have the ability to minimize orthodontic tooth movement. The aim of this study was to evaluate the effect of local administration of HA on orthodontic tooth movement and remodeling of alveolar bone related. The null hypothesis was that the local HA administration doesn't affect orthodontic tooth movement.

MATERIALS AND METHODS

This study was done according to the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines for animal studies (25) and after it was approved by the ethics committee, Faculty of Dentistry, Alexandria University in Egypt.

The minimal needed sample size was found to be 15 rabbits, it was calculated by adopting a power of 90% and level of significance 95% (α=0.05) (26). The standard deviation was determined according a previous study to be 0.548 (27).

Fifteen adult male white New Zealand rabbits were housed in separate cages, supplied with standard soft diet, tap water and standardized lighting in the animal house of Alexandria Medical Research Institute. The average weight of each rabbit was about 2.5 kg. All the experimental procedures took place under general anesthesia, the rabbits were anesthetized with intramuscular injection of Ketamine 35 mg/kg. (Alfasan, Woerden, The Netherlands) and Xylazine 5 mg/kg. (Xyla-ject, Adwia Pharmaceuticals, 10th of Ramadan City, Egypt).

A 12 mm Nickel-Titanium closed coil spring was fixed between each lower first premolar and the incisors delivering 100 gm of force (Fig 1). The force was measured using force gauge (Correx, Haag-Streit, Koniz, Switzerland) to standardize the applied force. The appliance was the same as that model used by Pithon and Ruellas (28) with modifications. Ligature wire was wrapped around the lower incisors in figure of eight tie, and in order to prevent any movement of the second premolar due to stretching of the gingival fibers during the first premolar movement, it was ligated to the molars using ligature wire and flowable composite.

Fig 1. NiTi closed coil spring was fixed between each lower first premolar and the incisors. The mandibular second premolar and first molar were ligated together.

A prospective randomized split-mouth experimental design was used, lower right and left sides were randomly assigned as control (A) and experimental (B) groups. For randomization, a computer-generated list of random numbers was used, while the researcher producing the randomization list was blinded to the treatment groups. In group A, injection of the placebo saline injection on day 0 while for group B, injection of HA 1% (2700 to 3200 kDa) (Viscoplus, Biomedical, Baumann GmbH, Frankfurt, Germany) on day 0, just after the appliance insertion. Then, the appliance was left for 21 days in place to achieve proper amount of tooth movement.

The injection was done intraligamentous and submucosal using a 0.5ml insulin syringe with a 31gauge ultra-fine needle (Insumed 31G x 8mm, Pic Solution, Artsana, Grandate, Italy). The main operator was blinded to which solution was being injected to each quadrant of the rabbits. The injection was performed mesially in the direction of tooth movement, so that injection was performed at 3 different points (mesiobuccal, mesial, and mesiolingual) into the mesial periodontal space of mandibular first premolar. The tip of the syringe was gently inserted until mild resistance was felt at the base of the gingival crevice and the solution was then delivered. For the submucosal injection, it was performed at 3 different points (mesiobuccal, mesial, and mesiolingual) mesial to the mandibular first premolar. The total HA injected was 120 µl for the experimental side and an equivalent amount of saline was injected in the control side.

Impressions for the mandibular posterior teeth were taken using sectional acrylic special trays which were fabricated on a pre-existing rabbit's dental cast. Light body injectable addition silicone vinyl polysiloxane impression material (Elite HD+ Light Body, Zhermack, Badia Polesine, Italy) was used for taking impressions for all the rabbits loaded in the previously described custom trays (Fig 2). Impressions were performed 2 times: on day 0 (baseline) and 21st day (end of experimental tooth movement) to allow the measurement of the magnitude of experimental tooth movement. All impressions were taken under anesthesia, and immediately poured with enhanced die stone (Elite Rock Dental Stone, Zhermack, Badia Polesine, Italy).

Fig 2. Light body addition silicone vinyl polysiloxane impression for the experimental teeth after 21 days of tooth movement.

The casts were scanned using a 3-D scanner (InEos X5, Sirona Dental Systems, Bensheim, Germany) to create 3-D digital models which were saved as .stl files. The amount of tooth movement was measured using Viewbox software (Version 4.0.1.7, Kifissia, Greece). Two planes were constructed, one tangent to the distal surface of first premolar and the other tangent to the mesial surface of the second premolar and both were perpendicular to the occlusal plane. The amount of tooth movement was equal to the distance between the 2 constructed planes (Fig 3). The method used for

measurement was similar to that used by Vieira et al (29) but with some modifications (30). The measurements were repeated to measure the intra-examiner reliability.

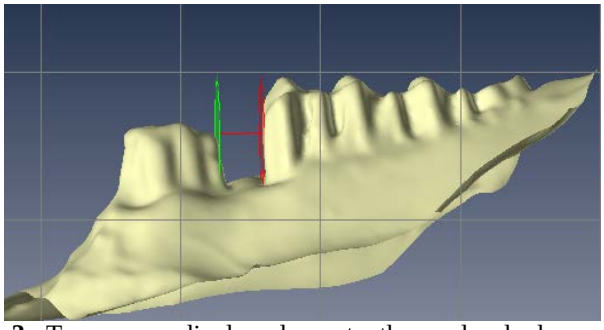

Fig 3: Two perpendicular planes to the occlusal planes and tangent to the distal and mesial surface of the first and second premolars respectively.

After the 21st day of the injection, rabbits were killed for histological analysis and the premolar region of both sides of the mandible were dissected, labelled and fixed and decalcified. Thin sections of 5 µm thickness were cut using rotary microtome. Random sections were stained with Haematoxylin and Eosin stain (H & E) for general examination under light microscope (OPTIKA, Via Rigla, Ponteranica (BG) - Italy) equipped with a 5-megapixel digital camera. From both groups A and B, sections were taken from the mesial side of the root of the mandibular first premolar and evaluated. Histologic analysis was done by single investigator in a blinded manner.

Statistical analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) software (Version 25, IBM, NY). Calibration on the measurement technique was done on 30 3D models (15 test and 15 control) and intra-examiner reliability was calculated with weighted Kappa. Normality was checked for all variables using Shapiro–Wilk test of normality. The comparison between the test and control was done using paired T-test. Significance level was set at P value ≤ 0.05.

RESULTS

Intra-examiner reliability was calculated with weighted Kappa = 0.87 and CI= 95% which indicated excellent agreement (31).

All the 15 rabbits tolerated well the experimental procedures. Normality was checked for all variables using descriptive statistics, Shapiro – Wilk test of normality. All variables showed normal distribution, so data was represented using means and standard deviations. Mean values and standard deviations calculated for the magnitudes of tooth movement are represented in **Table 1**.

Table 1: Mean values and standard deviations calculated for the magnitudes of tooth movement

	A	**B**
Mean ± SD	1.79 ± 0.17	1.61 ± 0.16
Mean Difference	0.17	
95% CI	0.10, 0.25	
P value	**<0.001***	

*statistically significant at p value <0.05

For the control group A, the measurements revealed 1.79 ± 0.17 mm of tooth movement, while in the experimental Group B (HA injected) the amount of tooth movement was only 1.61 ± 0.16 mm with statistically significant difference between both groups (P <0.001).

Light microscopic examination of specimens obtained from control group A revealed marked resorption of the bony wall on the mesial surface of mandibular first premolar. Rough alveolar bone surface with widespread intense resorption foci was observed. Many resorptive lacunae were seen containing multinuclear osteoclasts **(Figs. 4).** In the group B, the resorptive events were less dramatic. The bone surface facing periodontal ligament (PDL) showed areas of active bone resorption with resorptive lacunae, while other areas appeared relatively smooth. PDL fibers appeared compressed and well oriented. Some areas showed disorganized fibers **(Figs. 5)**.

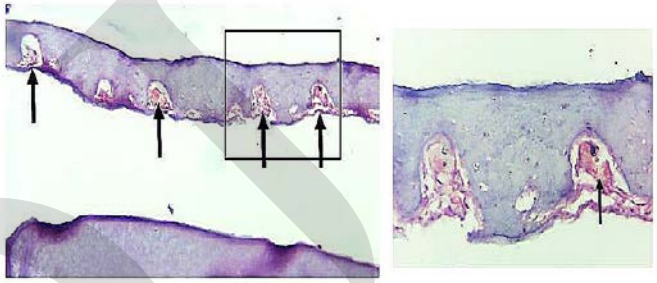

Fig 4: Light microscopic image of the mesial side of mandibular first premolar of control group (A) showing marked resorption on the mesial wall of the bone with multiple resorptive lacunae (arrows).

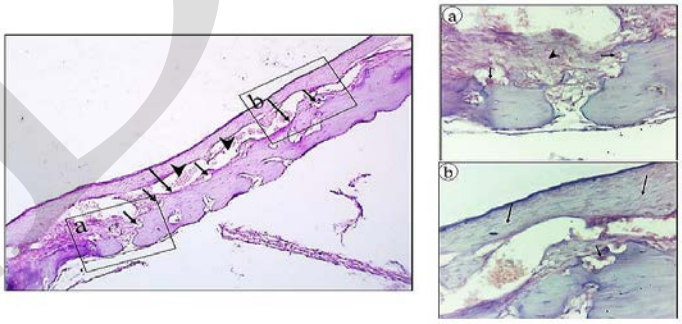

Fig. 5: Light microscope image of the mesial side of mandibular first premolar of the group (B) showing scattered areas of bone resorption (short arrows), while in other areas, the bone surface appeared relatively smooth (long arrows). Areas of interstitial spaces of periodontal ligaments (PDL) containing blood vessels can be seen (arrow heads). (H&E staining, x100). a & b are higher magnification (x400) showing osteoclasts in resorptive lacunae (short arrows). PDL fibres can be seen well oriented and slightly compressed (long arrows) or disorganized in other areas (arrow heads).

DISCUSSION

In our study we evaluated the effect of HA local injection on orthodontic tooth movement. Various experimental animals including rodents, monkeys, dogs and cats have been used in previous studies to study tooth movement (32, 33). Rats and rabbits are the animals of choice, because of their availability, to perform these studies. They are often considered the best animals for obtaining a good picture of bone changes under stress (34). In the current study, the rabbit model was chosen to test the hypothesis, as it is one of the most widely used models for studying bone remodeling. In comparison to rats, the rabbits are relatively easier in handling the intraoral orthodontic appliance, also they have wider periodontal width required for

the intraligamentous injection (35). Among various strains, New Zealand rabbits white strains are commonly used for research purposes as these strains' nature is less aggressive and usually have less health problems in comparison to other strains (34). Only male rabbits were chosen for the study to avoid any influence of sex hormones on tooth movement (36).

Split mouth design was preferred in this study to decrease the inter-animal variables, distal drift tendency of the premolars, the effect of growth and to help reduce the number of animals required for the study.

The orthodontic force used to induce orthodontic movement of mandibular premolars was approximately 100 gm, which is similar to the force used in previous studies evaluating tooth movement and relapse in rabbit model (6). Furthermore, the duration of active tooth movement was in accordance with the period used in these studies (6, 37).

Injection of HA was done intraligamental and submucosal to ensure the coverage of the whole area of the bone surrounding the tooth. Mendes et al (19), suggested a dose of 100 µl of 1% HA, which was effective to accelerate the healing process in tooth sockets of rats by stimulating the expression of osteogenic proteins. Sadikoglu et al (24), injected 50 µl of 3 % and 1.4% HA in the premaxillary suture of rats, so the dose used in our study, which was 120 µl of 1% HA, was believed to be sufficient to affect the bone biology.

The control group A yielded a magnitude of tooth movement equals (1.79 ± 0.17mm) which was higher than the experimental group B (1.61 ± 0.16mm). Local administration of HA resulted in a percentage inhibition of about 10% compared to the corresponding control group, this difference was statistically significant. From the point of view of anchorage these findings may be promising but the total inhibition of tooth movement still seems difficult to achieve.

Sadikoglu et al (24), evaluated the effect of HA on bone formation after orthodontic expansion and noted that the highest ratios of newly formed osteoblast and capillary cells were found in the high molecular HA group. Mendes et al (19), and Aslan et al (38), tested high molecular HA effect on healing process, the results showed more new bone formation and vascularization compared to controls. These previous results may explain the reduction in the magnitude of tooth movement in subgroup B. Also, the results could be attributed to the local anti-inflammatory effects of HA administration (16) and its ability to reduce the osteoclastic activity which is essential for orthodontic tooth movement.

The histological findings of this study also confirm the hypothesis that HA can hinder bone resorption and increase the ability of bone formation. Group B has less resorption activity with relatively more smooth bone surface, in comparison to the control group which showed the classical changes associated with the orthodontic tooth movement, including rough surface of the alveolar bone with widespread intensive resorption foci. These results conform with the findings of Mendes et al (19) and Aslan et al (38), and also the results support the theory that the interaction of HA and its receptor CD44 may restricts osteoblast-mediated osteoclastogenesis (39) .

Understanding the mechanism of osteoclast recruitment to compression sites after force application would have a profound effect on controlling orthodontic tooth movement. Rody jr et al (40) found that the highest percentage of osteoclastic cells located in the PDL in the first three days after orthodontic force application. We assumed that HA injection just after force application will have the maximum effect on osteoclastic activity. Decreasing the activity of the osteoclasts and consequently bone resorption may have direct effect to decrease orthodontic tooth movement.

Higher doses of HA and more frequent injections may have different effects on tooth movement and bone biology. Therefore, additional studies investigating the effect of higher doses of HA on bone and periodontal ligament could be beneficial and give better idea about the efficiency of HA injection.

CONCLUSION

There was statistically significant reduction in orthodontic tooth movement magnitude after local HA administration. Moreover, local administration of HA affected the bone remodeling, it reduced bone resorption activity.

Conflict of interest:

The authors declare that they have no conflicts of interest.

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