Dental Research Journal

Original Article

The effect of ovalbumin on orthodontic induced root resorption

Hosseinagha Aghili[1], Mohammad Danesh Ardekani[2], Seyed Amir Reza Fatahi Meybodi[1], Mohammad Hossein Toodehzaeim[1], Jalil Modaresi[3], Reza Mansouri[4], Ehsan Momeni[5]

[1]Departments of Orthodontics, [2]Oral Pathology, Shahid Sadoughi University of Medical Sciences, Yazd, [3]Endodontics, [4]Immunologist, [5]Department of Oral Medicine, Kerman University of Medical Sciences, Kerman, Iran

ABSTRACT

Background: This randomized trial was undertaken to investigate the effect of experimentally induced allergy on orthodontic induced root resorption.

Materials and Methods: A total of 30 Wistar rats were divided randomly into test and control groups. Starting from the first 3 days, the rats in the test group were injected intra-peritoneally by 2 mg ovalbumin as allergen and 0.5 mg Alume as adjuvant. Afterward only allergen was injected once a week. The control group was injected by normal saline. After 21 days, Wistar immunoglobulin E was measured and peripheral matured eosinophil was counted. A total of 50 g nickel-titanium closed coil spring was ligated between right incisor and first molar. All animals were sacrificed after 14 days. The mesial root of the right and left first molar was dissected in a horizontal plane. The specimens were divided into four groups considering whether force and/or ovalbumin was applied or not. Root resorption was measured and compared among these groups. Repeated measures analysis of variance (ANOVA), and Bonferoni tests were used to analyze the data. The level of significance was determined at 0.05.

Results: In general, the differences were insignificant ($P > 0.05$). As the only exception, the group in which both ovalbumin and force were applied had significantly more root resorption than the group in which neither force nor ovalbumin was applied ($P < 0.001$).

Conclusion: Allergy may increase the susceptibility to root resorption. Application of light force, periodical monitoring of root resorption and control of allergy are advisable.

Key Words: Allergy, orthodontic tooth movement, ovalbumin, rat, root resorption

Received: July 2012
Accepted: March 2013

Address for correspondence: Dr. Hosseinagha Aghili, Department of Orthodontics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
E-mail: hosseinaghily@gmail.com

INTRODUCTION

Orthodontic induced root resorption (OIRR) is one of the most prevalent sequelae of the treatment. It has been reported that 95% of adults receiving orthodontic treatment were affected by OIRR.[1,2]

Among the immunologic disorders, asthma and allergy are the most prevalent. The prevalence of allergy has been reported by world allergy organization to be as high as 30-40%.[3] In an Iranian population prevalence of 27.5% has been found.[4] There are evidences suggesting that the allergic disorders are increasing specially in the youngsters.[5]

The incidence and severity of OIRR can be influenced by several factors.[6-8] Owman-Moll and Kurol[9] investigated the possible risk factors of OIRR among which the allergy was the most prevalent. Although no significant statistical relation was found, it has been proven that immunological mechanism has a critical role in physiologic as well as the pathologic resorption of calcified tissues.[10] Davidovitch *et al.*[11] induced allergic asthma in a guinea pig model and suggested that chemical mediators may affect cell population and eventually the resorption process from the pressure side of the root.

Access this article online

Website: http//:drj.mui.ac.ir

Several studies have used ovalbumin for induction of allergy to investigate the effects of allergy in different situations. It has been shown to be a reliable allergen in animal studies.[12,13]

For the time being, there is no comprehensive study regarding the relationship between allergy and OIRR. This study was undertaken to investigate the effect of ovalbumin as an allergen on OIRR.

MATERIALS AND METHODS

Animals

A total of 30 male, 3-month-old 300-350 g weight Wistar rats were included in this study. The animals were housed paired in cages, with 12-h light-darkness cycle. Food and water were provided *ad lib*. This study is registered in Ethics Committee, Shahid Sadoughi University of Medical Sciences (#17/1/29606); and the National Research Council's Guidelines on caring and using laboratory animals were taken into consideration. The animals were quarantined for a week to get acclimated with the new situation before the study begins.

Allergy induction

The animals were divided randomly into test and control groups, each containing 15 rats. In the first 3 days, the rats in the test group were injected intra-peritoneally by 2 mg ovalbumin (Sigma-Aldrich co., Tokyo, Japan) as allergen and 0.5 mg Alume ($KAl[PO_4].[H_2O]_{12}$) as adjuvant. Afterwards only allergen was injected once a week. Meanwhile, the control group was injected by normal saline.

After 21 days, Wistar immunoglobulin E (IgE) was tittered by enzyme-linked immunosorbent assay (Shibayagi co. Ltd., Akrie, Japan) and peripheral matured eosinophils were counted. The exclusion criteria were as follows: For the test group, IgE <30 ng/ml, matured eosinophil <5% and animal mortality. For the control group, IgE ≥30 ng/ml, matured eosinophil ≥5% and animal mortality. No animal needed to be excluded from test or control group.

Application of orthodontic force

The orthodontic appliance was installed using the previously described method.[14] The animal was generally anesthetized by 25 mg/Kg of ketamine hydrochloride (Rotexmedica, Trittau, Germany) and 8 mg/Kg of xylazine (Rotexmedica, Trittau, Germany), administered intra-peritoneally. A total of 50 g nickel-titanium closed coil spring (Sentaloy®, GAC, Iship, NY) was ligated between the right incisor and first molar. The lower incisors were cut every 4 days to prevent the animal from incising over the appliance. Afterward the rat was nourished with soft pellet.

Groups and subgroups

The test group (O) where ovalbumin was administered was further divided into two subgroups: O-F; the right side molar to which the orthodontic force was applied and O-nF; the left side molar to which no force was applied. Likewise, in the control group (nO) where ovalbumin was not administered, the sub groups nO-F and nO-nF were formed by the same manner. In each subgroup, root resorption was measured at three levels: cervical (C), apical (A) and total (T), which is the mean of C and A [Figure 1].

During the course of this study, four animals died (two from test group and two from the control group). The obtained data from other animals were analyzed.

Histopathology

All animals were sacrificed by CO_2 overdose after 14 days of force application. The maxilla was

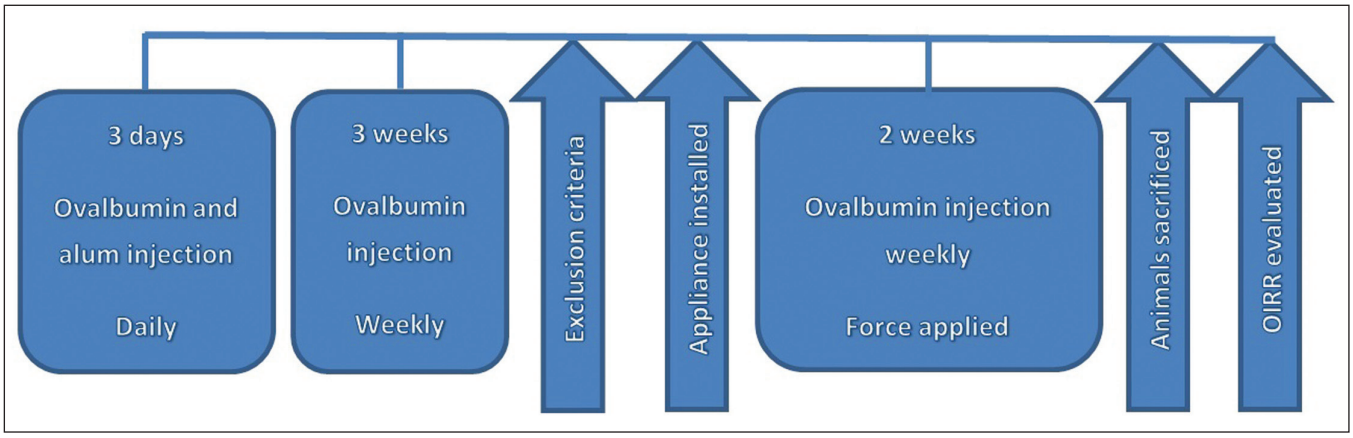

Figure 1: Flowchart of the study course

amputated, fixed in formalin 10% and decalcified by formic acid 10%.

Using a microtome (Leica®, Rm, Germany), the mesial root of right and left first molar was dissected in a horizontal plane. The dissections were done in two levels: cervical, defined as the first section containing alveolar bone and apical, at a distance of 1150 µ from the cervical level. At each level, three sections were obtained, 7 µ in thickness and 150 µ apart from each other. The sections were then stained by hematoxylin and eosin. Using a light microscope (Magnum®, Ceti co., GB), with ×40 magnification, images were shot by a digital camera (Dsch50 cybershot®, Sony, Japan).

The images were transferred to Photoshop software version 8 (Adobe, San Jose, CA), where the periphery of mesial and distal halves of the root was delineated in cervical and apical sections, respectively. On each periphery, the scalloped parts were considered as the resorptive lacunae regardless of the existence of cementoblasts. These parts were delineated separately [Figure 2]. The delineated curves were then transferred to AutoCAD 2009 (Autodesk Inc., San Rafael, CA), where the length of each was measured. Ten randomly selected sections were reevaluated by the same examiner. The *t*-test revealed a $P < 0.05$ and intra-examiner error was considered to be negligible.

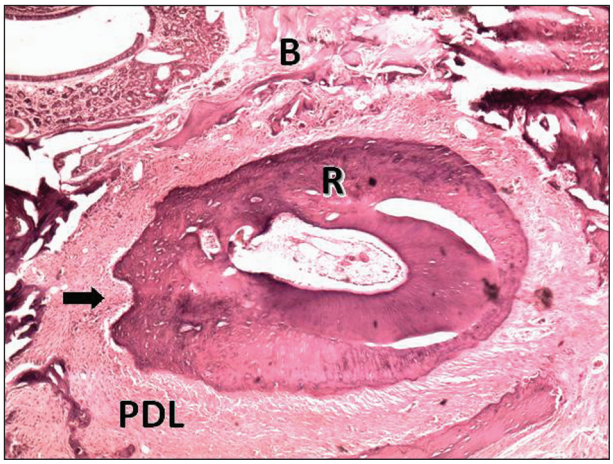

Figure 2: Histological section of the root at the cervical level. Note the resorption lacuna (black arrow). B: Bone; R: Root; PDL: Periodontal ligament

The amount of root resorption was defined as the mean length of the root periphery to the sum of the length of resorptive lacunae in the three sections obtained at the cervical or apical level.

Statistical analysis

The changes in weight were analyzed by repeated measures analysis of variance (ANOVA) and Bonfferoni tests. As for the amount of root resorption, ANOVA and Bonfferoni tests were used. The level of significance was determined at 0.05. Statistical analyses were performed by SPSS version 11.5 (SPSS corp., Chicago, IL).

RESULTS

Table 1 demonstrates the amount of root resorption in each group. The values varied from 16.5 ± 13.4 at apical level where neither force nor ovalbumin was applied, to 34.4 ± 6.1 in apical level where both force and ovalbumin were applied.

Table 2 demonstrates the comparison between groups. The O-F group has significantly more root resorption than nO-nF at all three levels; at the cervical level the difference was 13.6 ($P < 0.001$), at apical level the difference was 17.8 ($P < 0.002$) and totally the difference was 26.8 ($P < 0.001$). For all other groups, the differences were not significant ($P > 0.05$).

DISCUSSION

The rationales behind choosing the mesial root for evaluating root resorption were that this root is the largest one, from which better sections could be obtained and it is located centrally in the buccolingual dimension in the same plane as the force applied. In addition, it was used in several previous investigations.[15-19]

Table 1: The percentage of root resorption in each group (for the meaning of the abbreviations refer to section materials and methods, groups and subgroups)

Groups	O			nO		
	C	A	T	C	A	T
F	32.2±9.8	34.4±6.1	33.5±5.7	24.3±5.7	24.7±6.1	24.4±8.5
nF	23.4±6.2	24.5±9.0	23.9±7.2	18.6±6.7	16.5±13.4	17.2±16.6

Data were shown in the form of µ±SD

Table 2: Pairwise comparison of the groups; *P* values (for the meaning of the abbreviations refer to section materials and methods, groups and subgroups)

I	O-F			O-nF			nO-F		
J	I-J	*P* value	SE	I-J	*P* value	SE	I-J	*P* value	SE
nO-nF									
C	13.6	0.001*	3.2	4.7	0.935	3.2	5.7	0.263	2.7
A	17.8	0.002*	4.5	7.9	0.521	4.5	8.1	0.221	3.7
T	26.8	0.001*	6.2	14.4	0.161	6.2	11.0	0.254	5.2
nO-F									
C	7.8	0.132	3.2	−0.9	1.000	3.2	—	—	—
A	9.6	0.234	4.5	−0.2	1.000	4.5	—	—	—
T	15.7	0.098	6.2	−0.5	1.000	6.2	—	—	—
O-nF									
C	8.8	0.142	3.7	—	—	—	—	—	—
A	9.9	0.372	5.1	—	—	—	—	—	—
T	12.3	0.556	7.1	—	—	—	—	—	—

*Significance of the difference; SE: Standard error

In order to quantify root resorption, different methods have been used in various studies. Some have evaluated the root surface on a longitudinal sagittal section. While this method could reveal the defects from the cervical area to apical, it omits the buccal and lingual. In this study the buccolingual sections were obtained in cervical and apical region. One shortcoming of this method is the underestimation of the defect, since the section could simply pass through a narrow part of the defect. To overcome this issue, at each level, three parallel sections were passed with 150 μ intervals.[16]

To evaluate the spread of the defect, some investigators have used a grid and counted the lines passed through the defect.[16] Because of the ovoid geometry of the root, the buccal and lingual regions received fewer grid lines and thus the spread of defects in this area could be underestimated. In this study, software was used to delineate the border of the defect and measure its length, which could give a more realistic image. Another point is that in this study the ratio of defect to root surface was less than other studies, which used a grid.[16] Apart from the study parameters such as the drug used, one explanation lies in the fact that the defect located in mid-part has received more grid lines than the intact root surface in buccal and lingual parts.

Root resorption was evaluated on mesiocervical and distoappical regions. Since the tooth has undergone simple tipping movement in which the center of rotation is located in the mid-root region,[20] regarding the relation between OIRR and compression area in the periodontal ligament (PDL),[7-10,14] the reason for this selection becomes clear. A pilot study undertaken by the authors also justifies this.

Interestingly, in nF group, root resorption was also observed. This could be due to several factors: Occlusal trauma,[21] dentinal antibody[22] and the last but not least, the systemic side-effect of budesonide.

This study indicates an increasing tendency to root resorption in nO-nF, nO-F, O-nF and O-F groups; while the difference was significant only between nO-nF (where neither ovalbumin nor force was applied) and O-F (where both ovalbumin and force were applied)-(P value < 0.05).

Nishioka *et al.*[23] found allergy to be a strong risk factor for root resorption. Owman-Moll and Kurol,[9] also indicated the allergy as the most prevalent risk factor; although, no significant relation was found. The results of this study indicate that induction of allergy following intra-peritoneal injection of ovalbumin did not lead to a significant increase in OIRR compared to control group. This is in contrast with the abovementioned studies. This may be explained by the fact that these studies were case-controls and proof of allergy was based on self-declaration while the present study is a randomized controlled trial. The method of evaluating root resorption could be another reason.

Interestingly the difference was not significant between nO-F and nO-nF groups (P > 0.05), which means that the orthodontic force itself did not lead to an increase in root resorption. It has been shown that in the application of light force, the root resorption is negligible and should it occurs, the reparation is a common finding.[24]

As stated earlier, root resorption was greater in O-F group than in nO-nF. This may be due to the synergistic effect of allergy and inflammation produced by tooth movement. McNab *et al*.[25] stated that inflammation mediators produced in injured parts of the body can enter the PDL during the vasodilation period occurring in tooth movement. These mediators then can aggravate the inflammation around the root. This may increase the OIRR, as in persons suffering from asthma the first maxillary molar, which has roots in close proximity to the sinus is more prone to root resorption during the orthodontic treatment. Thilandeer *et al*.[26] believed that the presence of inflammatory cells affects the hardness of cementum and increases the vulnerability to OIRR. Rex *et al*.[27] stated that decrease in pH in the presence of inflammatory cells promotes osteoclastic activity, which could lead to root resorption.

This study has used 2 dimensional histological sections for assessment of root resorption. The method described in this study could give a more valid image than the previous studies, which used a grid; nonetheless 2 dimensional assessments have their own limitations and application of 3D methods such as micro computed tomography could be more accurate.

It is logical to suppose that other than the induced allergy, ovalbumin itself could primarily affect root resorption; this issue could not be clarified in this study. Using other allergens other than ovalbumin as well as primarily allergic models could shed more light to this.

CONCLUSION

The presence of allergy during the course of orthodontic treatment may increase the susceptibility to root resorption. Application of light force, periodical monitoring of root resorption and control of allergy are advisable.

ACKNOWLEDGMENTS

The authors wish to thank Torabinejad Research Center for their kind cooperation during the course of this investigation.

REFERENCES

1. Owman-Moll P. Orthodontic tooth movement and root resorption with special reference to force magnitude and duration. A clinical and histological investigation in adolescents. Swed Dent J Suppl 1995;105:1-45.
2. Ne RF, Witherspoon DE, Gutmann JL. Tooth resorption. Quintessence Int 1999;30:9-25.
3. World Allergy Organization [internet]. Milwaukee, WI: World Allergy Organization; 2011. Available from: http://www.worldallergy.org/publications/wao_white_book.pdf. [Accessed on 2013 Jan 22].
4. Varasteh A. Prevalence of allergic disorder among the population in the city of Mashhad, North East Iran. Public Health 2008;17:107-12.
5. Linge L, Linge BO. Patient characteristics and treatment variables associated with apical root resorption during orthodontic treatment. Am J Orthod Dentofacial Orthop 1991;99:35-43.
6. Levander E, Malmgren O. Evaluation of the risk of root resorption during orthodontic treatment: A study of upper incisors. Eur J Orthod 1988;10:30-8.
7. Wickwire NA, Mc Neil MH, Norton LA, Duell RC. The effects of tooth movement upon endodontically treated teeth. Angle Orthod 1974;44:235-42.
8. Odenrick L, Brattström V. The effect of nailbiting on root resorption during orthodontic treatment. Eur J Orthod 1983;5:185-8.
9. Owman-Moll P, Kurol J. Root resorption after orthodontic treatment in high-and low-risk patients: Analysis of allergy as a possible predisposing factor. Eur J Orthod 2000;22:657-63.
10. Anwar Waqar M. Prevalence of allergy and asthma in children of Islamabad, Pakistan. World Appl Sci J 2009;6:426-32.
11. Davidovitch Z, Goodwin S, Young-Guk P, Taverne AA. The etiology of root resorption. In: McNamara JA, Trotman CA, editors. Orthodontics: Management of Unfavorable Sequelae. Ann Arbor: Center for Human Growth and Development, University of Michigan; 1996. p. 93-117.
12. Young SH, Wolfarth MG, Roberts JR, Kashon ML, Antonini JM. Adjuvant effect of zymosan after pulmonary treatment in a mouse ovalbumin allergy model. Exp Lung Res 2013;39:48-57.
13. Anderson SE, Franko J, Kashon ML, Anderson KL, Hubbs AF, Lukomska E, *et al*. Exposure to triclosan augments the allergic response to ovalbumin in a mouse model of asthma. Toxicol Sci 2013;132:96-106.
14. King GJ, Fischlschweiger W. The effect of force magnitude on extractable bone resorptive activity and cemental cratering in orthodontic tooth movement. J Dent Res 1982;61:775-9.
15. Gonzales C, Hotokezaka H, Matsuo K, Shibazaki T, Yozgatian JH, Darendeliler MA, *et al*. Effects of steroidal and nonsteroidal drugs on tooth movement and root resorption in the rat molar. Angle Orthod 2009;79:715-26.
16. Verna C, Hartig LE, Kalia S, Melsen B. Influence of steroid drugs on orthodontically induced root resorption. Orthod Craniofac Res 2006;9:57-62.
17. Sekhavat AR, Mousavizadeh K, Pakshir HR, Aslani FS. Effect of misoprostol, a prostaglandin E1 analog, on orthodontic tooth movement in rats. Am J Orthod Dentofacial Orthop 2002;122:542-7.
18. Boekenoogen DI, Sinha PK, Nanda RS, Ghosh J, Currier GF, Howes RI. The effects of exogenous prostaglandin E2 on root resorption in rats. Am J Orthod Dentofacial Orthop 1996;109:277-86.

19. Leiker BJ, Nanda RS, Currier GF, Howes RI, Sinha PK. The effects of exogenous prostaglandins on orthodontic tooth movement in rats. Am J Orthod Dentofacial Orthop 1995;108:380-8.
20. Tanne K, Koenig HA, Burstone CJ. Moment to force ratios and the center of rotation. Am J Orthod Dentofacial Orthop 1988;94:426-31.
21. Nakamura I, Takahashi N, Sasaki T, Jimi E, Kurokawa T, Suda T. Chemical and physical properties of the extracellular matrix are required for the actin ring formation in osteoclasts. J Bone Miner Res 1996;11:1873-9.
22. Nyman S, Karring T, Lindhe J, Plantén S. Healing following implantation of periodontitis-affected roots into gingival connective tissue. J Clin Periodontol 1980;7:394-401.
23. Nishioka M, Ioi H, Nakata S, Nakasima A, Counts A. Root resorption and immune system factors in the Japanese. Angle Orthod 2006;76:103-8.
24. Paetyangkul A, Türk T, Elekdağ-Türk S, Jones AS, Petocz P, Cheng LL, *et al*. Physical properties of root cementum: Part 16. Comparisons of root resorption and resorption craters after the application of light and heavy continuous and controlled orthodontic forces for 4, 8, and 12 weeks. Am J Orthod Dentofacial Orthop 2011;139:E279-84.
25. McNab S, Battistutta D, Taverne A, Symons AL. External apical root resorption of posterior teeth in asthmatics after orthodontic treatment. Am J Orthod Dentofacial Orthop 1999;116:545-51.
26. Thilandeer B, Rygh P, Reitan K. Tissue reaction in orthodonrtics In: Graber TM, Vanarsdall RL, Vig KW, editors. Orthodontics: Current Principles and Techniques. St. Louis, Mosby; 2005. p. 145-219.
27. Rex T, Kharbanda OP, Petocz P, Darendeliler MA. Physical properties of root cementum: Part 6. A comparative quantitative analysis of the mineral composition of human premolar cementum after the application of orthodontic forces. Am J Orthod Dentofacial Orthop 2006;129:358-67.

How to cite this article: Aghili H, Ardekani MD, Meybodi SR, Toodehzaeim MH, Modaresi J, Mansouri R, *et al*. The effect of ovalbumin on orthodontic induced root resorption. Dent Res J 2013;10:630-5.

Source of Support: Vice chancellery of research, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. **Conflict of Interest:** None declared.