Original Article

Lack of Interleukin 1-beta Expression Following Orthodontic Induced Root Resorption

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Abstract

Objective: To determine the effect of orthodontic tooth movement on the expression of interleukin-1β mRNA in rats using RT-PCR.

Materials and Methods: Sample consisted of eighteen 8-week-old male Wistar rats. The right maxillary first molar of each animal was protracted using an orthodontic protraction appliance. The left maxillary first molar received no treatment and was assigned as the control group. On day 21, all rats were sacrifice and divided in two equal groups. The first group, group (A), was histologically evaluated for the presence and size of potential resorptive lacunae. The second group, group (B), was investigated using RT-PCR in order to determine IL-1β mRNA expression.

Results: Measurements revealed that the mean tooth movement was 0.23 mm in group A and 0.24 mm in group B. The mean depth of the resorptive lacunae was 0.17×10^{-11} mm^2 in the control group and 4.9×10^{-11} mm^2 in the intervention group (control group: left maxillary first molars; right maxillary first molars were divided to group A & B, histologic study of group A assures the existence of resorptive lacunae and its extent relative to control group). The difference between the two groups was statistically significant ($p<0.05$). The RT-PCR evaluation showed no significant differences in IL-1β mRNA expressions of resorptive lacunae between the treated and untreated groups.

Conclusion: Although interleukin1-beta is the most potent stimulator of bone resorption and mediator of inflammatory response, the present study showed that the IL-1beta mRNA was not expressed more significantly in root resorption lacunae of the treated molars relative to the control group.

Keywords: Interleukin-1 beta, Gene Expression, Root Resorption, Orthodontics

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Introduction

Orthodonticaly induced root resorption is the most frequent unwanted side effect of orthodontic tooth movement that is unavoidable (1). The sterile inflammatory process initiated by force application is the major cause of root resorption and bone remodeling (1, 2). Many experiments have dealt with problems associated with root resorption. Root resorption is a multicausal problem; for instance, systemic (3-5), iatrogenic, and age (6, 7) factors have been considered.

One of the iatrogenic causes is orthodontic treatment. The duration of orthodontic force, whether constant or intermittent, and its direction play important roles (8, 9).

Interleukin-1 is a multifunctional cytokine with a wide variety of activities. IL-1α and IL-1β are two active peptides encoded by two separate gene products. They have identical activities and potentials. Gowen et al. showed that IL-1 is the first polypeptide mediator of immune cell function that regulates bone resorption (10). Canalis showed that IL-1 regulates bone formation (11) and demonstrated that this cytokine, as an osteoclast activating factor (OAF), is a major contributor to bone resorption. Lorenzo et al. reported that IL-1 is the most potent stimulator of bone resorption and promoter of prostaglandin synthesis in bone (12).

Tooth movement occurs following the presence of prostaglandin E as a second messenger. Seifi et al. showed that the administration of prostaglandin E following application of orthodontic force significantly increases orthodontic tooth movement and causes simultaneous remarkable root resorption (13). Lorenzo et al. showed that an IL-1 induced

increase in prostaglandin synthesis is a stimulator of bone resorption activity (12).
Proinflammatory cytokines, such as interleukin-1, TNF-alpha and IL-6 are believed to play a role in biological processes involved in the course of orthodontic tooth movement and especially root resorption (14, 15).
The interleukin-1 family (IL-1 alpha, IL-1 beta, and IL-1 RA) has been shown to be a potent stimulus for bone resorption. According to previous studies, the external apical root resorption (EARR) phenomena associated with IL-1 in particular, is caused by peptide hormones which act as signaling substances in the immune system and amplify osteoclast differentiation which in turn affects resorption (15, 16). Previous studies have mentioned that the presence of elevated levels of IL-1 beta in the periodontal tissue and gingival crevicular fluid of patients after orthodontic tooth movement also plays a role in orthodontic tooth movement (17-20). Inflammatory process occurs in an aseptic environment during application of mechanical force to teeth following palatal expansion. IL-1beta has been shown to be present in the process of bone resorption in patients undergoing orthodontic tooth movement (21, 22) and it also can be a contributing factor to root resorption. Al-Qawasmi et al. showed that absence of IL-1 beta cytokine activity did not affect baseline root resorption (1). Gulden et al. showed that there is no genetic predisposition for EARR caused by the IL-1 beta allele (23); however, they did note a correlation between this IL-1 variant and EARR. Furthermore, in a previous clinical finding reported that IL-1 beta cytokine is a significant factor in root resorption associated with orthodontic tooth movement (24).
The aim of this study was to determine the relation of orthodontic tooth movement induced root resorption to IL-1 beta gene expression in resorptive lacunae.

Materials and Methods

Eighteen -week-old Wistar male rats weighing 300g have been used in this experimental split-mouth study. During the study, rats were fed a soft food diet ad libitum. Our animal selection and orthodontic protocol were approved by Shahid Beheshti University of Medical Sciences' School of Dentistry Institutional Ethics Committee. In this experiment, right first upper molars of the rats were protracted with orthodontic appliance and taken as the case group, while the left maxillary molars which had no treatment were considered as the control group.

Anesthesia and orthodontic appliance installation

On the first day, all the rats underwent operation under sterile conditions. The animals were sedated by an IP injection of ketamin 44 mg/kg (Proke-Davis) and xylazine hydrochloride 2% (0.1 mg/kg). A slot was prepared with the aid of a handpiece bur on the distobuccal portion of the maxillary incisors. Orthodontic appliance including a 9 mm long NiTi closed coil spring (Dentaurum, 0.008 × 0.02 inch) was tightened to the first molar posteriorly and to the upper incisor anteriorly using a steel ligature wire. No-mix composite was utilized for wire fixation on the teeth. Orthodontic force (60 g) was applied to the teeth for 21 days to produce the protracting movement of the maxillary first molar.

Specimen preparation

After being treated for twenty one days, the animals were euthanized with an overdose of pentobarbital. They were divided into two equal groups (A & B) and their resulting orthodontic tooth movements (OTMs) between the right maxillary first and second molars were measured by using calibrated gauges with an accuracy of 0.05 mm. Furthermore, the molars from experimental (OTM) side of group A (9 teeth) were histologically examined, and those of group B (9 teeth) were investigated for RT-PCR.
The left maxillary first molars of both groups served as the control group and did not receive protraction force. This group was also divided into two equal parts and examined for the histology and RT-PCR of the root surfaces.

Histologic evaluation

The maxillae were dissected out, divided into two halves which were fixed in neutral buffered formalin and decalcified with formic acid 10% for 7 days. They were then dehydrated in an ascending series of alcohol rinses. In order to increase their translucency, they were also placed in 50% and 100% methyl salicylate solutions for 2 and 5 hours respectively. These prepared blocks were then embedded in paraffin.
Serial sections with the thickness of 5µm in a mesio-distal direction, parallel to the long axis of the mesial root of the first molar were cut with a Microm HM 335 rotary microtome (Microm International GmbH, Walldorf, Germany). The central section of each tooth which shows the maximum surface of its root was considered the measuring criteria for all specimens. Selected sections were stained with haematoxyline-eosine (HE).

Images were captured from the mesial portion of each first molar under a microscope with a Fujix Hc-300Zi digital camera (Nikon, Japan). Midmesial sections of the roots were chosen and the mean surface areas of the resorptive lacunae in the mesial portion of the first molar teeth were histomorphometrically measured.

The RT-PCR evaluation

The mesial root of each first maxillary molar with the alveolar bone was extracted by means of rongeur forceps. The root and bone were detached and placed separately in normal saline in -20°C. The tooth and bone were placed in EDTA 5% for twenty four hours, and were sonicated to achieve complete lyses. One milliliter of RNX plus buffer was added to each specimen. After staying in room temperature for 10 minutes, 200 µL of chloroform was added to the mixture. It was then centrifuged for 15 minutes in 4°C at 12000 rpm. The upper phase was moved to another microtube and pure ethylic alcohol, in equal volume to the upper phase, was added to the specimen. The RNA sediment was rinsed with 100µL alcohol (70%) and was centrifuged for 2 minutes at 12000 rpm. The RNA sediment was solved in 25 µL of diethylpyrocarbonate (DEPC) treated water. The specimens were then heated to 70°C in order to open the probable RNA loops before reaction reverse transcription.

cDNA synthesis

The transcriptase reaction was prepared with the following: 1 µg of RNA, 1×RT buffer, 0.3 mM dNTP, 100 units RNasine, 200 units of reverse transcriptase enzyme, DEPC treated water up to 20 µl. The reaction was incubated at 42°C for one hour.

PCR amplification

The PCR reaction included the following: total synthesized cDNA, 0.2 mM dNTP, 1.5 mM MgCl2, 40 pmol each of forward and reverse primers, 1× PCR buffer, 1.25 units of Taq DNA polymerase (CinnaGen, Iran), and ddH_2O to give a final volume of 50 µL. PCR cycling parameters included an initial 5 min preincubation at 94°C for 5 minutes, with incubation cycles consisting of denaturation at 94°C for 30 seconds, annealing temperature of 65°C for 60 seconds and extension at 72°C for 30 seconds. 30 cycles were repeated followed by a final incubation at 72°C for 5 minutes.

Nested PCR

The nested PCR was prepared as the PCR. In the presented study, PCR product was used instead of cDNA. The PCR product was electrophoresed in 3% agarose gel, stained with ethidium bromide and observed under a UV transilluminator devise. The PCR product for IL1-β was considered as 297 nucleotides.

Results

Orthodontic tooth movement observation

After comparing the degree of orthodontic tooth movements (OTMs) between groups A and B, it was concluded that a significant difference did not exist. Acccording to normal distribution of data, statistical test was student t-test and p=0.872, statistical package of SPSS 11.5. It was hence deduced that both groups would experience the same phenomenon from the root resorption perspective. Consequently, group A was assigned for histologic evaluation and group B was assigned for RT-PCR evaluation. Furthermore, the tooth movements between control and experimental groups were significantly different because the control group did not receive any protraction force and was only used for histologic and RT-PCR examinations.

The results of the OTM experiment have been shown in table 1 and the examination of root resorption in table 2. According to the Mann-Whitney U test the magnitude of root resorption in group A and control groups were significantly different ($p<0.001$). It shows that a significant orthodontic tooth movement is capable of creating root resorptive craters and lacunae (Fig 1).

Table 1: Orthodontic tooth movement in the histologically examined group A and the RT-PCR evaluated group B in millimeters

Groups	Count	Mean	SD	Maximum	Minimum
A	9	0.23	0.0559	0.35	0.15
B	9	0.24	0.0846	0.4	0.15

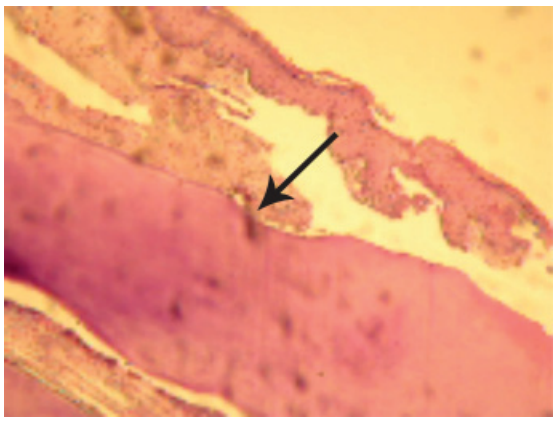

Fig 1: Arrow shows a resorptive lacuna on the root following significant orthodontic tooth movement.

Table 2: Measurement of root resorption in group A and control group in square millimeters

Root Resorption	Mean $\times 10^{-11} mm^2$	SD $\times 10^{-11} mm^2$	Std. Error mean$\times 10^{-11}$	Mean difference$\times 10^{-11}$	t test results
Group A (n=9)	4.9	1.63	0.543	4.73	0.0001
Control group (n=9)	0.17	0.51	0.0168		

Table 3: IL-1β index comparison between the experimental and control groups using Wilcoxon signed rank test

Groups	Count	Positive amounts	Negative amount	Mean rank	Sum of ranks	z	P-value
Experiment	5	2	3	0.00	0.00	-1.414	0.157
Control	5	4	1	1.5	3.00		

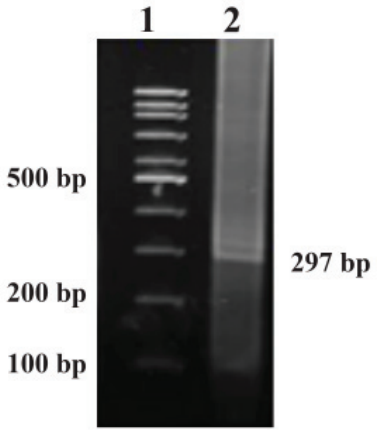

Fig 2: PCR product electrophoresis in 3% agarose gel. Lane 1= DNA ladder marker, lane 2= A 297 bp fragment as the IL-1 beta PCR products.

RT-PCR Evaluation

According to the RT-PCR for the IL-1β mRNA gene expression in the root resorptive lacunae of group B and root craters or erosions in the control group, the authors conclude that there is no significant difference between the groups in relation to IL-1β gene expression (Fig 2).

Discussion

The investigation data regarding the IL-1 beta mRNA expression did not correlate with resorptive lacunae production, however further cellular and molecular analysis of larger animal groups is needed to confirm the irrelevance of IL-1beta genetic expression and root resorption during orthodontic tooth movement.

In the presented experiment, histological changes were considerably pronounced after force application to allow for the evaluation of the root for amount of resorption and IL-1β expression. This study was focused on the correlation between IL-1 beta expression and root resorption following orthodontic force application, and showed that there was no fundamental connection between specific IL-1β alleles and sporadic external root resorption.

The present investigation data fail to confirm the association reported by Al-Qawasmi et al. between external apical root resorption and IL-1β expression (1). The aforementioned authors divided the control rats into two groups and IL1-beta in two groups of treated and untreated. They supported findings that the IL-1 cytokine was a significant factor in root resorption associated with orthodontic tooth movement. Knock out mice have enabled scientists to understand the function of a gene in its absence. At the end of an inbred line, the knocked-out gene is expressed in the complete absence of its viable copy of the gene.

Gene expression hypothetically may take place on the root surface or precisely in the resorptive lacunae. By knocking out the gene, its expression would be inhibited in a complete manner. Consequently, it is an intervening factor that is eliminated during research.

In the presented study, the resorptive phenomenon was surveyed locally and gene expression was investigated in the resorptive lacunae in an effort to eliminate the role of IL-1 in the bone. In their study, Al-Qawasmi et al. express the inequality of root resorption between wild and knockout experimental groups without mentioning the degree of orthodontic tooth movement (1). The present study, the molars in the control and experimental groups were matched because a decreased rate of alveolar bone catabolic bone remodelling may result in the prolonged compression of root against the alveolar bone.

Gulden et al. demonstrated a correlation between IL-1α polymorphism and EARR (23); however,

they did not find IL-1β as a predisposing factor in EARR which shows consistency with our experiment. It is worthy to mention that osteopontin, osteonectin, cytokeratin 8 and granulocyte colony-stimulation factor (G-CSF) are other predisposing factors for root resorption (23).

Alhashimi et al. performed an in situ hybridization in order to measure the messenger RNA expressions of IL-1β, IL-6, and TNF-α at 3, 7, and 10 days after application of orthodontic force on the maxillary first molars of 12 rats. They showed that the mRNA for IL-1 and IL-6 had reached their maximal expression on day three and finally they concluded that these proinflammatory cytokines may play important roles in bone resorption after application of orthodontic force (15).

Ogasawara et al. evaluated the in situ expression of receptor activator of nuclear factor -κB ligand (RANKL), receptor activator of nuclear factor -κB (RANK), osteoprotegerin, interleukin-1 β (IL-1β) and tumour necrosis factor α (TNFα) in osteoclasts of rat periodontal tissue (25). They discovered that an autocrine mechanism of RANKL-RANK exists in the osteoclast, and is increased in pathological conditions. They also observed that in osteoclasts, the autocrine mechanism of IL-1 beta and TNF alpha is evident under pathological conditions. Hence, they concluded that the autocrine mechanisms seem to both pathologically and physiologically regulate the osteoclast function in both physiological and pathological conditions (25).

Similarity between resorptive processes of bone and root, from a genetic point of view, is supported by RANKL mRNA expression during root resorption (26) and osteopontin expression during OTM (27). Given the genetic proximity between these two hard tissues i.e. bone and tooth, it should be emphasized that they are different tissues and some genetic diversity exists among them leading to different genetic expression in a similar situation.

Conclusion

Although interleukin-1 beta is the most potent stimulator of bone resorption and mediator of inflammatory response, the presented study shows that the IL-1beta mRNA is not expressed significantly higher in root resorption lacunae of the experimental group relative to the control group. In quantitative measurements, the IL-1beta expression in root resorption currently reveals no indication of its possible predisposing role in resorptive lacunae production.

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