Original Article

Effects of Interferon-Gamma on Bone Remodeling during Experimental Tooth Movement

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ABSTRACT

Objective: To determine the effects of interferon-gamma (IFN-γ) on bone remodeling during orthodontic tooth movement.

Materials and Methods: Thirty adult male Sprague Dawley rats were randomly categorized into five groups. IFN-γ was administered in three different doses (0.01, 0.02, and 0.05 μg/20 μL) and the remaining two groups served as control. Mandibular first molars were moved mesially by means of Ni-Ti closed coil springs in all groups. The results were evaluated histomorphometrically, and parameters of trabecular bone volume (BV/TV), trabecular bone number (Tr.N), and trabecular separation (Tr.Sep) were observed at the interradicular bone area of the mandibular first molars.

Results: Increases in BV/TV and Tr.N and decreases in Tr.Sep revealed the antiosteoclastic activity of IFN-γ.

Conclusion: IFN-γ administration may be useful clinically for anchorage control.

KEY WORDS: Interferon-gamma; Rat; Local administration; Tooth movement; Bone remodeling

INTRODUCTION

Orthodontic tooth movement requires remodeling of alveolar bone, and cytokines have been suggested of major importance in bone remodeling. As in other connective tissues, these effects are mediated via paracrine, autocrine, and endocrine mechanisms. The principal cytokines that have been shown to affect skeletal tissues include factors previously described as monokines or lymphokines, colony-stimulating factors, prostaglandins, and differentiation factors.[1–3] The T helper 1 cytokine interferon-gamma (IFN-γ) is one of them.

Interferons were originally described in 1957 as an activity found in the supernatant of virally infected cells that directly ''interfered'' with viral replication.[4] These proteins have been classified into two types based on structural and functional criteria and the stimuli that elicits their expression. Type I interferons are primarily induced in response to viral infection and have been categorized into two subgroups: IFN-α and IFN-β. Type II interferons, known as IFN-γ, are synthesized primarily by defined subsets of T lymphocytes and natural killer cells after activation with immune and inflammatory stimuli.[5–7]

IFN-γ has multiple important functions in immunoregulation. Impaired IFN-γ production or increased IFN-γ levels were determined in various diseases, and IFN-γ has been used after organ transplantations as an immunosuppressive.[7–11] Although IFN-γ inhibits osteoclast formation, it has been successfully used in the treatment of osteopetrosis.[12]

The phenomenon of bone resorption is very complex and has been coordinated by cellular and hormonal factors at in vivo conditions. IFN-γ inhibits bone resorption and has an inhibitory effect on osteoclasts at the level of differentiation.[11–15] IFN-γ is an important control mechanism in osteoclastogenesis.[12,16–19]

Previous studies revealed that IFN-γ inhibits bone resorption stimulated by interleukin (IL)-1 and blocks collagenase production caused by parathyroid hormone, prostaglandin E2, 1.25(OH)2 vitamin D3, IL-1, tumor necrosis factor-α (TNF-α), and epidermal

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growth factor.[20–22] On the other hand, various investigations have introduced conflicting results suggesting that IFN-γ modulates the function and activities of osteoblasts, osteoclasts, and chondrocytes, resulting in bone resorption, leukocyte function, and in defects of bone and cartilage.[12,16]

The functions of IFN-γ in periodontal tissue have been investigated in several studies. The results of these investigations suggested that IFN-γ plays an important role in the regulation of periodontal tissue destruction and periodontal remodeling during orthodontic tooth movement.[23,24] Although IFN-γ is well known in general medicine, a review of the literature showed that IFN-γ was not evaluated extensively in orthodontics. The purpose of this investigation was to determine the effects of IFN-γ on bone during orthodontic tooth movement.

MATERIALS AND METHODS

The experiment was carried out according to the guidelines for the use of experimental animals of Gülhane Military Medical Academy. The study consisted of 30 adult male Sprague Dawley rats of approximately the same age with an average weight of 250 g. The rats were randomly categorized into five equal groups with six rats in each group. They were fed a standard pellet diet with tap water at libitum and were adapted to a 12:12-hour light:dark cycle.

The rats were anesthetized with a combination of xylazine hydrochloride (HCL) (10 mg/kg) (Alfazyne 2%, Egevet, Izmir, Turkey) and ketamin HCL (90 mg/kg) (Alfamine 10%, Egevet, Izmir, Turkey) before the application of the springs. The same chemicals were also used as a lethal dose before sacrifice. Isoflurane inhalation anesthetic (10 mg/kg) was used before various procedures. An orthodontic force was administered to all groups. Ni-Ti closed coil springs (0.305 × 0.915 mm) exerting 80 g of reciprocal force were applied bilaterally between the mandibular first molars and incisors. A prophylactic antibiotic (Colicillin (0.1 ml/kg) (100 mg/ml Ampicillin + 250.000 IU/ml Colistin Sulphate, Egevet, Izmir, Turkey) was administered once to prevent infection that could result from trauma during application of springs. The force was measured with a gauge, and the springs were not reactivated during the experiment.

Recombinant rat IFN-γ (rRIFN-γ) (Biological Ind, Kibbutz, Beit Haemek, Israel) was dissolved in 0.9% sodium chloride (NaCl) and prepared at three different concentrations: 0.01, 0.02, and 0.05 μg/20 μL. In the first group, 0.01 μg/20 μL rRIFN-γ was administered once a day with 80 g of orthodontic force. In the second and third groups, 0.02 and 0.05 μg/20 μL rRIFN-γ were administered, respectively, with the same sequence and dose. The last two groups were evaluated as control groups. In the fourth group, 0.9% NaCl solution was administered with the same amount and prescription (20 μL/24 h).

The experimental solutions were injected into the mesiobuccal subperiosteal area adjacent to the left and right lower first molars. Only orthodontic force was administered in the fifth group. The rats were monitored during the experiment and were sacrificed on day 7 of the experiment. The mandibles were dissected out and placed in 0.1 M phosphate-buffered 2.5% glutaraldehyde solution for fixation.

Bone Histomorphometry

Histological sections were prepared according to the "Cutting-Grinding Technique."[25] The mandibles were dehydrated with increasing ethanol concentrations. After dehydration, specimens were exposed to plastic infiltration with an increasing mixture of ethanol-methyl methacrylate. The plastic-infiltrated tissue slices were placed in embedding molds, Technovit 7.200 VLC (Heraeus-Kulzer GmbH & Co KG, Hanau, Germany) was added, and final polymerization process was accomplished.

The polymerized tissue blocks were sectioned with a band saw to 100 μm thickness and transferred to a microgrinding system, and a tissue thickness of 10–20 μm was obtained. The sections were agitate in 10% H_20_2 for 5 minutes, rinsed in water, wiped dry, and stained in toluidine blue.

All sections were evaluated with an Olympus microscope (Olympus Optical Co, Tokyo, Japan), and 4× images were transferred to a computer and the WinTAS (Trabecular Analyze System, version 1.2.9) program (Steve Paxton, University of Leeds, Leeds, UK) (Figures 1 and 2).

Measurements were performed in the interradicular area of the first molars (cervical, middle, and apical). Measurements were repeated two times and the mean values were calculated. All measurements and calculations were done according to the American Society for Bone and Mineral Research nomenclature and guidelines.[26] Trabecular bone volume (BV/TV), trabecular bone number (Tr.N), and trabecular separation (Tr.Sep) were measured by using the software package developed for bone histomorphometry.

Statistical Method

The statistics were performed by using an SPSS 10.0 (SPSSFW, SPSS Inc, Chicago, Ill) statistical package. Descriptive statistics were shown as mean ± standard deviation (Table 1). The differences between all groups were investigated by Kruskal-Wallis test, and P values ≤.05 were evaluated as statistically

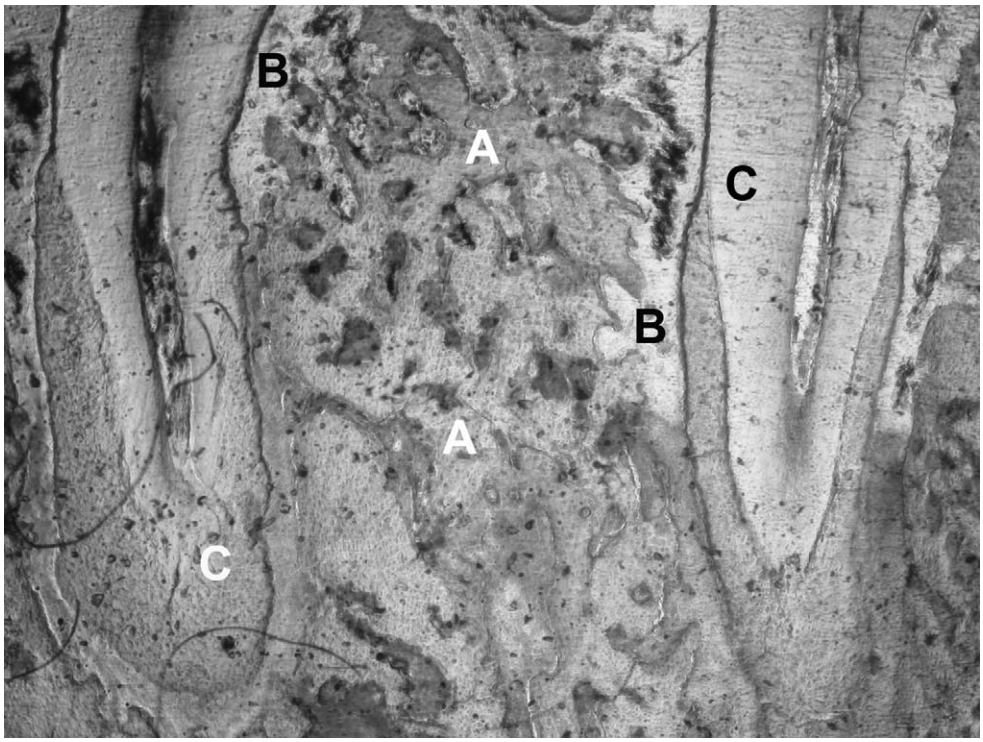

Figure 1. Histological section of group 3. (A) Trabecular bone. (B) Bone resorption area. (C) Root of the first molar.

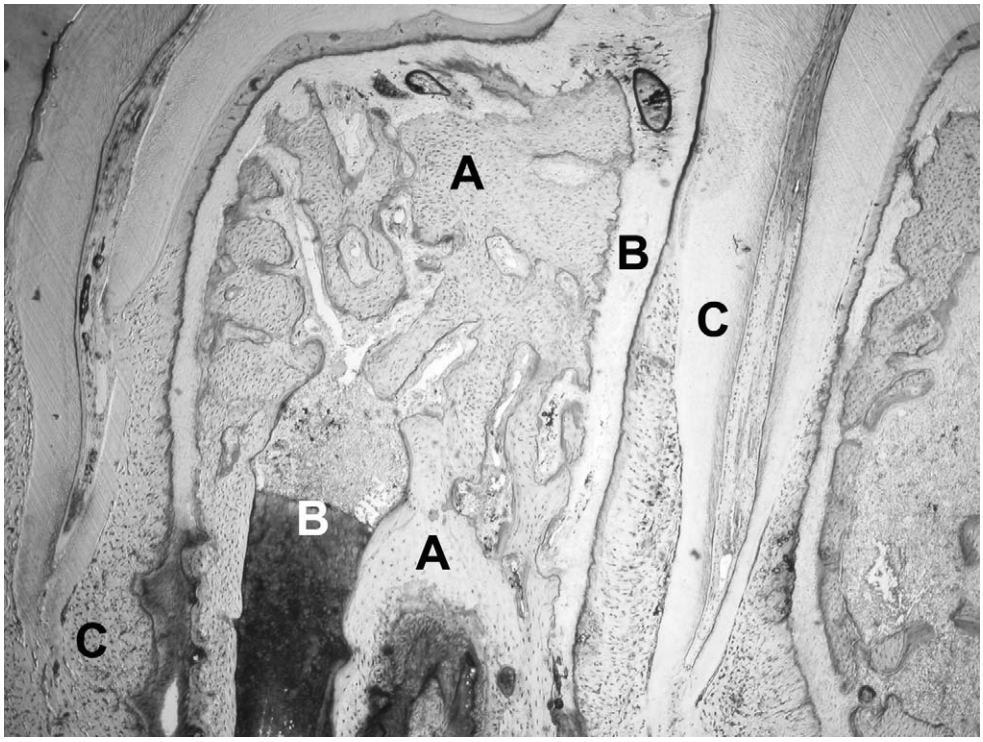

Figure 2. Histological section of group 5. (A) Trabecular bone. (B) Bone resorption area. (C) Root of the first molar.

Table 1. Descriptive Statistics of Trabecular Bone Volume (BV/TV), Trabecular Bone Number (Tr.N), and Trabecular Separation (Tr.Sep) for All Groups

	BV/TV		Tr.N		Tr.Sep	
Group	Mean	SD	Mean	SD	Mean	SD
1	27.600	9.469	23.379	6.210	39.578	9.402
2	27.701	9.191	23.373	7.384	39.293	16.469
3	30.120	8.935	24.891	7.230	34.901	7.860
4	17.165	8.654	19.494	5.887	54.300	13.937
5	18.225	12.783	19.168	8.661	64.800	12.994

Table 2. Kruskal-Wallis Test of All Groups[a]

	χ^2	*P*	Significance
BV/TV	11.462	.020	*
Tr.N	4.854	.303	NS
Tr.Sep	21.980	.000	***

[a] BV/TV indicates trabecular bone volume; Tr.N, trabecular bone number; Tr.Sep, trabecular separation; and NS, not significant.
* $P < .05$; *** $P < .001$.

Table 3. Comparison of Intragroup Differences for Trabecular Bone Volume (BV/TV), Trabecular Bone Number (Tr.N), and Trabecular Separation (Tr.Sep)[a]

Groups		*Z*	*P*	Significance
1 and 2	BV/TV	−0.456	.684	NS
	Tr.N	−0.454	.684	NS
	Tr.Sep	−0.227	.853	NS
1 and 3	BV/TV	−0.643	.526	NS
	Tr.N	−0.529	.631	NS
	Tr.Sep	−1.173	.247	NS
2 and 3	BV/TV	−0.532	.631	NS
	Tr.N	−0.454	.684	NS
	Tr.Sep	−0.076	.971	NS
4 and 5	BV/TV	−0.114	.912	NS
	Tr.N	−0.076	.971	NS
	Tr.Sep	−1.740	.089	NS

[a] NS indicates not significant.

significant. The differences between IFN-γ and control groups were determined by Mann-Whitney *U*-test. *P* values ≤.05 were evaluated as statistically significant.[27]

RESULTS

Statistically significant differences were observed in BV/TV ($P < .05$) and Tr.Sep ($P < .001$) parameters when all groups were compared with each other (Table 2). In the comparison of the intragroup differences, any statistically significant alterations were observed in the IFN-γ and control groups (Table 3).

In the comparison of groups 1 and 4, an increase in BV/TV and a decrease in Tr.Sep were statistically significant ($P < .05$), and any statistically significant difference was observed in Tr.N ($P > .05$). The decrease in Tr.Sep was significant ($P < .001$) between groups 1 and 5, whereas the differences in Tr.N and BV/TV were not significant ($P > .05$) (Table 4).

Table 4. Comparison of Between-Group Differences for Trabecular Bone Volume (BV/TV), Trabecular Bone Number (Tr.N), and Trabecular Separation (Tr.Sep)[a]

Groups		*Z*	*P*	Significance
1 and 4	BV/TV	−2.307	.019	*
	Tr.N	−1.134	.280	NS
	Tr.Sep	−2.192	.029	*
1 and 5	BV/TV	−1.815	.075	NS
	Tr.N	−1.059	.315	NS
	Tr.Sep	−3.250	.000	***
2 and 4	BV/TV	−2.050	.043	*
	Tr.N	−1.514	.143	NS
	Tr.Sep	−2.081	.035	*
2 and 5	BV/TV	−1.898	.063	NS
	Tr.N	−0.908	.393	NS
	Tr.Sep	−2.875	.003	**
3 and 4	BV/TV	−2.575	.009	**
	Tr.N	−1.815	.075	NS
	Tr.Sep	−3.102	.001	***
3 and 5	BV/TV	−2.158	.029	*
	Tr.N	−1.589	.123	NS
	Tr.Sep	−3.556	.000	***

[a] NS indicates not significant.
* $P < .05$; ** $P < .01$; *** $P < .001$.

When groups 2 and 4 were compared, the increase in BV/TV and decrease in Tr.Sep were found significant ($P < .05$), but the change in Tr.N was not significant ($P > .05$). A statistically significant decrease in Tr.Sep ($P < .01$) was observed in the comparison of groups 2 and 5, whereas the differences in BV/TV and Tr.N were not significant ($P > .05$) (Table 4).

In the comparison of groups 3 and 4, the increase in BV/TV and decrease in Tr.Sep were statistically significant ($P < .01$ and $P < .001$), but the changes in Tr.N were not significant ($P > .05$). In the comparison of groups 3 and 5, the increase in BV/TV ($P < .05$) and decrease in Tr.Sep ($P < .001$) were significant, whereas the differences in Tr.N were not significant ($P > .05$) (Table 4).

DISCUSSION

The local microenvironment is central to the regulation of osteoclastic activity, and studies show that different factors may influence the rate of orthodontic tooth movement via various biomediators.[1–3] Because the short cycle in female rats causes hormonal variations, our study was carried out with male rats.[28,29] In previous studies, orthodontic force was exerted by closed coil springs between the molars and incisors or elastics between the first and second molars of the rat.[30–33] The mechanics used for force application resulted in the creation of retention points that caused gingival inflammation.[30–35] As a result, mediators that are effective in bone resorption and osteoclastic activ-

ity were excreted.[36] As easy as it is designed, the application of force mechanics leads to the risk of trauma, edema, and infection. To eliminate these probabilities, one prophylactic dose of antibiotic was administered to all rats.

The absence of investigations of the local administration of IFN-γ in experimental tooth movement led us to take advantage of other studies that used systemic or local applications of the cytokine and to adjust the doses for our experiment. In the literature, intravenous (IV), intramuscular (IM), and subcutaneous (SC) administrations to obtain systemic effects and cell culture applications were found. The half life of 100 g/m² IFN-γ was 38 minutes IV, 2.9 hours IM, and 5.9 hours SC. Accumulations and adverse effects were not observed after use of a single dose per day for 12 consecutive days. Dose-dependent inhibition of osteoclast differentiation was demonstrated previously, and the mean dose for IFN-γ in rat was 5 MU kg/d.[8,11,13–15,27,37] In light of previous studies and with the aim for local administration, the dose adjustment for IFN-γ was below the systemic doses. Additionally, we intended to observe the dose-dependent effects of IFN-γ; therefore, three different doses of the cytokine were administered.

When the outcomes of the WinTAS analysis were interpreted, the increase in BV/TV was 60.79% in group 1, 61.38% in group 2, and 75.47% in group 3 when compared with the control group 4. Similarly, in the comparison of the IFN-γ groups with group 5, the increase was 51.44% in group 1, 51.99% in group 2, and 65.27% in group 3. Although all alterations in the measured parameters were not statistically significant, these findings are in accordance with the results of in vitro studies denoting the primary effect of IFN-γ over osteogenic cells and osteoblasts and a decrease in bone resorption.[20–22]

In the comparison of the parameter Tr.N, the increase was 19.93% in group 1, 19.90% in group 2, and 27.69% in group 3 compared with group 4 and 21.97% in group 1, 21.94% in group 2, and 29.86% in group 3 compared with group 5. When the Tr.Sep values of IFN-γ groups were compared with control groups, the decreases in Tr.Sep were 27.11% in group 1, 27.64% in group 2, and 35.73% in group 3 compared with group 4 and 38.92% in group 1, 39.36% in group 2, and 46.14% in group 3 compared with group 5. Increases in Tr.N and decreases Tr.Sep in the IFN-γ groups reinforced the argument that IFN-γ affects the rate of bone resorption. This could be through the stimulation of the osteoblastic cells or the inhibition of osteoclastic cells.

Alhashimi et al[23] investigated the effects of IFN-γ, IL-4, and IL-10 by in situ hybridization and measured the protein levels of IFN-γ by immunohistochemistry. Induction of IFN-γ at both messenger RNA and protein levels was significantly higher on the experimental side than on the contralateral control side on day 3. The signal gradually became stronger on day 7 and remained high on day 10. The authors suggested that IFN-γ may be involved in periodontium remodeling during orthodontic tooth movement because of its potential immunoregulatory roles. These results support our findings.

Saito et al[38] investigated the effects of IL-1α and IL-1β, TNF-α, and IFN-γ on Prostaglandin E (PGE) and cyclic adenosine monophosphate (cAMP) production by periodontal ligament fibroblasts. They concluded that fibroblasts respond to all the cytokines with a dose- and time-related increase in the levels of PGE and cAMP. These cytokines may regulate the function of the fibroblasts in physiological remodeling of the periodontium, as well as in inflammatory reactions.

Cornish et al[16] evaluated the effect of IL-18 on mature osteoclast activity and reported that IL-18 did not induce IFN-γ production by primary osteoblasts. However, IFN-γ had the opposing action to IL-18 and inhibited primary osteoblast cell proliferation. T cell–derived factors may regulate osteoclast formation and may also influence osteoblast and chondrocyte activities. These results are in contrast with our determinations.

Key Jr et al[12] used IFN-γ for long-term therapy in patients with osteopetrosis and increases in bone resorption and hematopoiesis and found an improvement in leukocyte function. It seems paradoxical at first, but when the immunoregulatory function of IFN-γ is considered, the effect of IFN-γ in osteopetrosis did not conflict with our results. As is well known, in osteopetrosis an inactivation of osteoclasts and a deficiency in regular bone resorption are the points at issue. Overall, IFN-γ performed its regulation process by activating the osteoclasts and inducing the bone resorption. These results are in concordance with our observations and the conclusions of other investigations relating the immunoregulatory properties of IFN-γ.[1,10,16,18]

Ukai et al[24] investigated the balance between IFN-γ and IL-4–bearing cells in human inflamed gingiva by immunohistochemistry. A low ratio of IL-4–bearing cells to IFN-γ–bearing cells was involved in the destruction of periodontal tissue. These results substantiated again the reality that IFN-γ is a cytokine increasing the cellular and humoral immune response in inflammation.[6,24]

The reason for bone apposition instead of resorption in this investigation was because of orthodontic tooth movement rather than infection. In the absence of infection, IFN-γ represents its primary effect as antiosteoclastic activity. The statistical increases in BV/TV and decrease in Tr.Sep between groups 3 and 5, the

insignificant but important increases BV/TV and Tr.N, and the decreases in Tr.Sep parameters between the IFN-γ and control groups emphasize a dose-dependent effect of IFN-γ. The increase in dose resulted in an increase in bone apposition and osteoblastic activity or inhibition of osteoclastic activity.

CONCLUSIONS

- The antiosteoclastic activity of the groups injected with IFN-γ was greater than in the control groups.
- Our data suggest that IFN-γ is involved in bone remodeling during orthodontic tooth movement, which strongly suppresses osteoclastogenesis.
- IFN-γ administration may be clinically useful for anchorage control.

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