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ORIGINAL ARTICLE

Enhancement of orthodontic anchorage and retention by the local injection of strontium: An experimental study in rats

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KEYWORDS

Orthodontic anchorage;
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Abstract *Objectives:* To examine the clinical and histological effects of locally injected strontium on the anchoring unit of a rat model of an experimental relapsed tooth movement.

Materials and methods: Thirty-six 10-week-old male Wister rats were randomly divided into two groups of 18 animals that were then randomly divided into three subgroups of six animals corresponding to three observation periods: T1 = 1 week, T2 = 2 weeks, and T3 = 3 weeks. In the first experiment, both the right and left maxillary first molars were moved buccally with a standardized expansive spring. Strontium chloride solution was injected every 2 days into the subperiosteal area buccal to the left maxillary first molar (the experimental side). The right-sided first molar was injected with distilled water as a control. In the second experiment, maxillary first molars were moved buccally with the spring. After 3 weeks, the spring was removed. Two days before the spring removal, strontium chloride was injected into the palatal side of left-sided maxillary first molar and distilled water was injected into the palatal side of the right-sided maxillary first molar as in experiment 1.

Results: At the end of the experimental period, significant levels of inhibition were noted in terms of both tooth movement and relapse movement in strontium-injected sides. Histological examinations showed that strontium enhanced the number of osteoblasts and reduced the number of osteoclasts.

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Conclusion: The local injection of strontium can inhibit the degree of experimental and relapsed tooth movement in a rat model.

1. Introduction

Headgears, mini-screw implants, intra-oral elastics and other types of anchorage are routinely used in orthodontic practice. Removable acrylic retainers, fixed retainers and even surgical interventions are sometimes used to prevent post-orthodontic relapses. All of these anchorages and retentive measures have stood the test of time in providing the best possible treatment outcomes, with the exception of some limitations such as difficulties in their manufacture, applications and patient compliance. The use of drugs as an adjunct for orthodontic treatment has been proposed, either to accelerate the speed of orthodontic movement or by retarding undesired tooth movement (Igarashi et al., 1994; Atack et al., 2007; Keles et al., 2007). Orthodontic tooth movement is induced by the controlled application of mechanical forces, which results in bone resorption by osteoclasts on the compression side of teeth, and bone formation by osteoblasts on the opposing (tension) side. Recent research advances have suggested that biological modulators that inhibit osteoclasts could be used as novel adjunctive approaches to orthodontic treatment (Igarashi et al., 1994; Nishimura et al., 2008). Fresh insights into molecular details of osteoclastic bone resorption have provided new opportunities for the identification of agents that can selectively modulate osteoclast activity (Masella and Chung, 2008).

Within the last decade, several effective medical compounds have been introduced for the treatment of osteoporosis, including strontium ranelate. Because of the well-known beneficial effects of strontium ranelate on bone formation and remodeling, in addition to its low rate of side effects, this drug is considered to be the first choice for treating osteoporosis (Canalis et al., 2007). Strontium ranelate is an oral anti-osteoporotic agent that is selectively concentrated in positions of active bone formation and exerting both anti-catabolic and anabolic effects on bone cells by being able to stimulate bone formation via osteoblasts and to inhibit bone resorption by osteoclasts. It is, therefore, referred to as a "dual action bone agent" (Cesareo et al., 2010). The active ingredient in strontium ranelate is strontium (Sr), which has key effects on bone metabolism. Strontium (Sr) is an alkaline earth trace metal cation that has a high affinity for hydroxyapatite (Pors Nielsen, 2004). This bone-building action works by downregulating osteoclastogenesis by modulating the RANKL/OPG balancing system.

The RANKL/OPG balancing system is important in regulating RANKL and OPG in periodontal tissues and for osteoclastogenesis and therefore it is critical to regulate bone remodeling during orthodontic tooth movement (Kim et al., 2007). Hence, the present experimental study used local injections of strontium as a biological bone cell modulator to investigate both clinical and histological effects on experimental and relapsed tooth movements in rats.

2. Materials and methods

2.1. Laboratory animals

Fifty 10-week-old male Wister rats weighing 250–300 g were purchased from the animal house of the National Center of Drug Control and Research in Baghdad, Iraq and were treated according to the ethical guidelines of the animal care staff at the Center. The animals were housed under normal laboratory conditions and adapted to a standard 12/12 h light/dark cycle at 25 °C with constant humidity. The animals were fed with a diet of commercially manufactured standard laboratory pellets and water *ad libitum*.

2.2. Study design and sample categorization

The study was performed with the permission of the Animal Care staff at the National Center of Drug Control and Research in Baghdad, Iraq. The design of the study included two experiments (Fig. 1). Experiment 1 aimed to examine the clinical and histological effects of a local injection of strontium on experimental tooth movement of the anchor unit. Experiment 2 aimed to examine the clinical and histological effects of strontium injection on relapsed tooth movement. Eight animals were not included in the statistical analysis due to death during anesthetic and spring dislodgment. An initial pilot study was conducted using six animals and the main study consisted of 36 10-week-old male Wister rats that were randomly divided into two groups of 18 animals for each experiment. For both

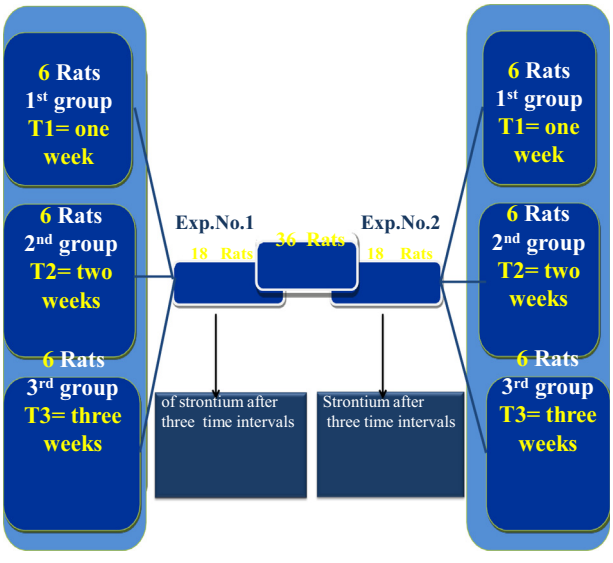

Figure 1 Flow chart summarizing the study design and sample categorization.

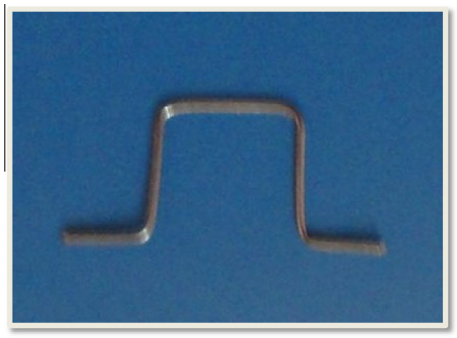

Figure 2 Expansive spring.

experimental groups, these 18 animals were randomly divided into three subgroups of six animals that corresponded to three observation periods: T1 = 1 week, T2 = 2 weeks and T3 = 3 weeks. In both experiments, a uniform standardized expansive spring was set into the mouth of each animal between the right and left maxillary first molars to move them buccally (Fig. 2). Each spring was made of 0.012-inch nickel–titanium and manufactured by the Iraqi Ministry of Science and Technology; the springs were similar to those described by Igarashi et al. (1994), Adachi et al. (1994), Nishimura et al. (2008) and Miyoshi et al. (2001). The spring initially generated an average expansive force of 20 g on each side. All laboratory procedures were established after general anesthesia was induced with an intramuscular injection of ketamine (50 mg/kg body weight) and the muscle relaxant, orbarcaine 2%, at a dose of 5 mg/kg body weight. The two drugs were mixed at the ratio of 2:1 (ketamine:orbarcaine).

2.3. Experiment 1

All animals in the three subgroups received an expansive force (Fig. 3) without any adjustments during the experimental period. After a precise impression was made with silicon materials and spring placement in anesthetized animals, 0.25 ml of strontium chloride solution (concentration, 240 mg/ml) was injected with an extra-fine needle syringe locally at the sub-periosteal area of the buccal side of the left-sided maxillary first molar, which served as the experimental side. On the other side, 0.25 ml of distilled water was injected at the sub- periosteal area of the buccal side of the right maxillary first molar, which served as the control side in all groups. The injection was repeated every 2 days under general anesthesia for the entire study period. After the injection was completed, the animals were returned to their cages and allowed to recover. After 1 week, the expansive springs were removed from the first group, study models were made for tooth movement measurements after force applications were made for this period, the animals were scarified humanely and specimens were taken for histological examination. The method was repeated for the second and third groups at their respective time points.

2.4. Experiment 2

All procedures in the experiment 1 were repeated in these animals, except the springs were left for 3 weeks before the maxillary first molars were moved buccally (Fig. 4). After 3 weeks, the animals in all three time point groups were anesthetized and 0.25 ml of strontium chloride solution (240 mg/ml) was injected locally at the palatal side of the left maxillary first molar, which served as the experimental side. Then, 0.25 ml of distilled water was injected at the palatal side of the right maxillary first molar, which served as the control side. This procedure was performed 2 days before the springs were removed. After the removal of the spring, study models were prepared. The injections continued every 2 days under general anesthesia for the whole study period. After 1 week, the animals in the first group were anesthetized, impressions were taken and study models were made for relapse tooth movement measurements for this period. The animals were then humanely sacrificed and specimens were taken for histological examination. The method was repeated for the second and third groups at their respective time points.

2.5. Tooth movement measurements

The occlusal view of a precise stone model of the control (right) and experimental (left) maxillary molars was magnified 40 times using a camera (Leica) and the outline was traced. To orientate the measurements, the contours of the palatal cusps of the second and third molars of these tracings were then superimposed onto those of the second and third molars of tracings from the pretreatment stone model. The distance between the crests of the mesiopalatal cusps of the first molars before and after tooth movement was measured with electronic

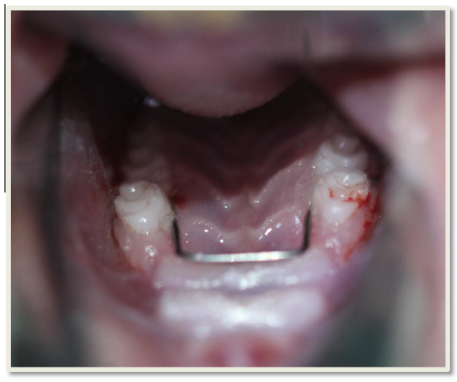

Figure 3 Expansive spring in place within the rat maxilla.

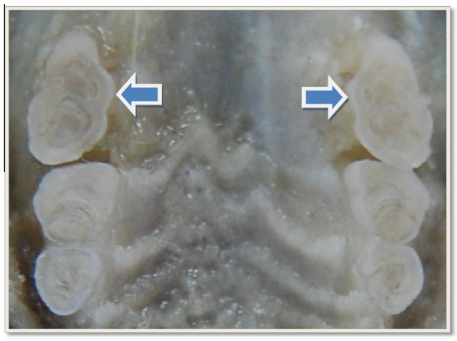

Figure 4 Maxillary first molars moved bucally.

digital caliper and represented the amount of tooth movement according to the method described by Adachi et al. (1994).

2.6. Histology

Upon the completion of each experimental time intervals, the anesthetized animals were sacrificed humanely. The entire maxilla was dissected, immediately fixed in 10% neutral buffered formalin solution overnight and then decalcified in 10% formic acid for 15–20 days. Each maxilla was dissected into two halves, the experimental and control sides, and each half included the three maxillary molars according to the method described by Adachi et al. (1994). The specimens were then dehydrated and embedded in paraffin. Each block was cut into serial 5 μm-thick cross-sections with a microtome. The sections were stained with hematoxylin and eosin and observed under light microscopy. Periodontal tissues of the buccal roots of the maxillary first molars were examined in serial cross-sections of the molars at the level of the bifurcation. The periodontal tissues of the buccal roots area were divided into pressure and tension sides based on the mesiodistal axis of the root (Fig. 5a), and the number of osteoclasts and osteoblasts within the bone of the pressure side of the experimental and control molars were counted at a magnification of ×400. Osteoblasts were identified based on their morphology as being cuboidal, with a plump configuration, large nuclei, and localized on the bone or osteoid surface (Fig. 5b). Tartrate-resistant acid phosphatase (TRAP) staining was used to identify osteoclast cells. Osteoclasts were identified as large multi-nucleated cells stained with eosin, containing round nuclei and located immediately adjacent to the bone surface (Fig. 5c).

2.7. Statistical analysis

Both descriptive and inferential statistical analyses were used. The paired sample *t*-test was used to identify differences between control and experimental sides. A p-value $\leqslant 0.05$ was considered to be statistically significant.

3. Results

3.1. Animal condition

No appreciable inflammatory response was observed at the local injection site in any of the animals in the study. The application of orthodontic force did not affect the body weights of rats.

3.2. Results of experiment 1

As shown in Tables 1–6, the highest mean value of tooth movement was found in the control side of the third group. No significant difference was found in the mean of tooth movement in the first group (p-value = .058) compared to either the second (p-value = .000) and third (p-value = .000) groups. Regarding osteoblast number, the highest mean value was found on the experimental side of the third group. The differences in the mean osteoblast number were not significant (p-value = .085) with in the first group but were significant in the second

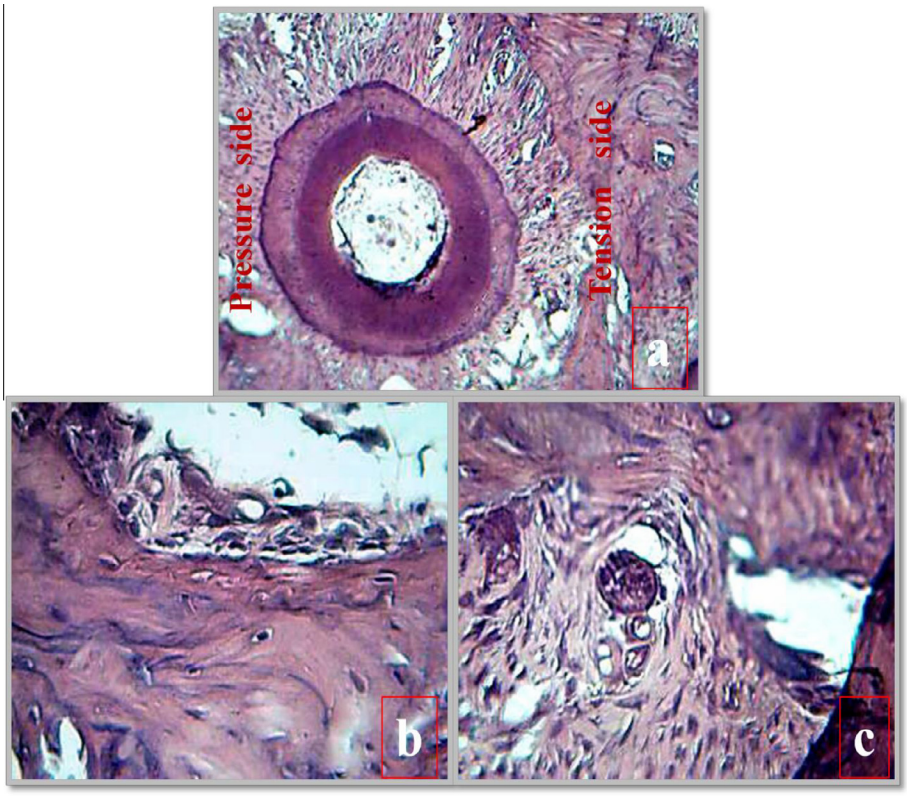

Figure 5 (a) Microscopic observation of periodontal tissues of the mesiobuccal root of the rat molar (magnification ×100). (b) Microscopic view of osteoblast cells (magnification ×400) (c) Microscopic view of osteoclast cells in Howship's lacunae (magnification ×400) (H&E staining).

Table 1 Descriptive information on experimental tooth movement measurements in the three groups.

Groups	variables	Min.	Max.	Mean	SD	SE
1st Group	Tm R side	.25	.31	.28	.021	.008
	Tm L side	.23	.30	.27	.027	.011
2nd Group	Tm R side	.49	.53	.51	.015	.006
	Tm L side	.30	.35	.32	.021	.008
3rd Group	Tm R side	.69	.71	.69	.007	.003
	Tm L side	.30	.36	.33	.025	.010

$N = 6$, Tm = tooth movement measured in mm, R = right, L = left.

Table 2 Experimental – control difference of the tooth movement measurements and the *t*-value for experimental – control difference with its degrees of freedom and *p*-value.

Groups	Mean $_{Difference}$	SD $_{Difference}$	SE $_{Difference}$	*t* value	*p* value
1st Group	.011	.011	.004	2.44	.058
2nd Group	.190	.015	.006	30.04	.000
3rd Group	.363	.022	.009	39.53	.000

df = 5.

Table 3 Descriptive information on osteoblast numbers in the three groups.

Groups	variables	Min.	Max.	mean	SD	SE
1st Group	O.B R side	7.20	9.50	8.30	.126	.051
	O.B L side	8.80	9.10	8.98	.116	.047
2nd Group	O.B R side	10.10	10.40	10.25	.104	.042
	O.B L side	11.90	12.10	12.00	.063	.025
3rd Group	O.B R side	14.07	14.47	14.20	.150	.061
	O.B L side	15.80	16.40	16.05	.197	.080

$N = 6$, O.B = osteoblast number, R = right, L = left.

Table 4 Experimental – control difference of the osteoblast numbers and the *t*-value with its degrees of freedom and *p*-value.

Groups	Mean $_{difference}$	*SD* $_{difference}$	*SE* $_{difference}$	*t* value	*p* value
1st Group	−.68	.780	.318	−2.14	.085
2nd Group	−1.75	.083	.034	−51.23	.000
3rd Group	−1.84	.075	.030	−60.09	.000

df = 5.

(*p*-value = .000) and third (*p*-value = .000) groups. Regarding osteoclast number, the highest mean value was found on the control side of the third group. Differences in the mean number of osteoclasts were found to be not significantly different with in the first (*p*-value = .063) and second (*p*-value = .077) groups, but were significantly different in the third (*p*-value = .002) group.

3.3. Results of experiment 2

As shown in Tables 7–12, the highest mean value of relapsed tooth movement was found in the control side of the third group. No significant difference in the mean tooth movement was observed with in the first group (*p*-value = .076) but significant differences were found in both the second (*p*-value = .000) and third (*p*-value = .000) groups. Regarding osteoblast number, the highest mean value was found on the experimental side of the third group. There were significant differences in the mean number of osteoblasts in all three groups (*p*-value = .004, .000 and .000 respectively). Regarding osteoclast number, the highest mean value was found on the control side of the third group. No significant differences were found in the mean number of osteoclasts with in the first (*p*-value = .085) and second (*p*-value = .146) groups but a significant difference was found in the third group (*p*-value = .000).

4. Discussion

The undesired movements of anchor teeth and relapses of movement of previously moved teeth, are major clinical problems in orthodontics. A promising strategy for maintaining anchorage may be the use of biological inhibitors of osteoclastic bone resorption. Biological modulators that modulate an osteoclast activity could be administrated locally to control undesired tooth movement at anchor units or systemically to enhance post-treatment stability (Keles et al., 2007; Masella and Chung, 2008). Therefore, the present study aimed to examine the clinical and histological effects of locally injected strontium on experimental and relapsed tooth movement in rats and its ability to enhance orthodontic anchorage and retention via its bone-building capacity. Strontium chloride (SrCl2) was used instead of strontium ranelate, as it exhibits very similar effects from animal studies. Furthermore, it can be easily dissolved in distilled water, which makes its laboratory use and application more reliable. The concentration of strontium chloride used was extrapolated from doses recommended by Ammann et al. (2004).

4.1. Experiment 1

At the end of the first week, no significant differences were observed between the control and experimental sides in terms of the rate of experimental tooth movement. This finding was consistent with those of Adachi et al. (1994) and could be explained by the fact that initial tooth movement within

Table 5 Descriptive information on osteoclast numbers in the three groups.

Groups	Variables	Min.	Max.	Mean	SD	SE
1st Group	O.C R side	.60	.70	.65	.054	.022
	O.C L side	.30	.65	.49	.128	.052
2nd Group	O.C R side	.90	1.50	1.26	.287	.117
	O.C L side	.80	1.10	.98	.116	.047
3rd Group	O.C R side	2.10	3.30	2.86	.413	.168
	O.C L side	1.80	2.20	1.96	.136	.055

$N = 6$, O.C = osteoclast number, R = right, L = left.

Table 6 Experimental – control difference of the osteoclast number and the *t*-value with its degrees of freedom and *p*-value.

Groups	Mean $_{difference}$	SD $_{difference}$	SE $_{difference}$	*t* value	*p* value
1st Group	.15	.16	.06	2.38	.063
2nd Group	.28	.31	.12	2.22	.077
3rd Group	.90	.35	.14	6.26	.002

df = 5.

the first week was primarily due to the compression of the periodontal ligament rather than from bone remodeling, in accordance with the hypotheses of King and Fischlschweiger (1982) and Igarashi et al. (1994). Concerning the number of osteoblasts and osteoclasts, there were also no significant differences at the end of the first week. This may be explained by the suggestion of Okumura (1982) and Nakamura et al. (2001), whereby bone remodeling can only start after the removal of necrotic or hyalinized tissue by phagocytic cells, which occurs during the first week after force application.

At the end of the second week, the rate of tooth movement was reduced significantly in the experimental side and the number of osteoblasts was significantly increased in the pressure sides of the experimental molars. At the same time, there were no significant differences in the number of osteoclasts. The inhibition in tooth movement may be explained by the ability of strontium to increase osteoblast replication, osteoblast differentiation and bone matrix synthesis and mineralization, in accordance with the findings of Canalis et al. (1996), Barbara et al. (2004), Marie (2005), Seeman et al. (2006) and Ammann et al. (2007). According to Pavlin et al. (2000), during bone formation, the proliferation of osteoblasts has a smaller role compared to a marked increase in the differentiation of individual cells; furthermore, the bone matrix synthesis and mineralization forms as a result of osteoblast function. Therefore, further histological and immunohistochemical studies are required to examine the exact changes in the osteoblast activity at that time. Moreover, the inhibition of tooth movement, despite the insignificant changes in osteoclast numbers, may be explained by the results of Lees et al. (2001) and Manolson et al. (2003).

Previous studies have demonstrated that the size and activity of osteoclasts are also related to the ability of the individual osteoclast to resorb bone, according to the conclusions drawn by Mentaverri et al. (2005) and Hurtel-Lemaire et al. (2009).

Table 7 Descriptive information on the measurements of the relapsed tooth movement in the three groups.

Groups	Variables	Min.	Max.	Mean	SD	SE
1st Group	Tm R side	.23	.28	.26	.017	.007
	Tm L side	.23	.28	.25	.017	.007
2nd Group	Tm R side	.58	.61	.59	.012	.004
	Tm L side	.45	.48	.46	.013	.005
3rd Group	Tm R side	.68	.71	.69	.011	.004
	Tm L side	.48	.50	.49	.008	.003

$N = 6$, Tm = Tooth movement measured in mm, R = right, L = left.

Table 8 Experimental – control difference of the relapsed tooth movement measurements and the t-value with its degrees of freedom and p-value.

Groups	Mean $_{difference}$	SD $_{difference}$	SE $_{difference}$	t value	p value
1st Group	.01	.010	.004	2.23	.076
2nd Group	.12	.005	.002	55.90	.000
3rd Group	.20	.005	.002	91.67	.000

df = 5.

Table 9 Descriptive information on osteoblast numbers in the three groups.

Groups	Variables	Min.	Max.	Mean	SD	SE
1st Group	O.B R side	10.30	10.80	10.50	.178	.073
	O.B L side	10.88	11.17	11.00	.112	.045
2nd Group	O.B R side	13.00	13.50	13.28	.231	.094
	O.B L side	14.20	14.80	14.53	.233	.095
3rd Group	O.B R side	16.00	16.60	16.30	.219	.089
	O.B L side	17.50	18.00	17.66	.225	.091

$N = 6$, O.B = osteoblast number, R = right, L = left.

Table 10 Experimental – control difference of the osteoblast numbers and the t-value with its degrees of freedom and p-value.

Groups	Mean $_{difference}$	SD $_{difference}$	SE $_{difference}$	t value	p value
1st Group	−.50	.245	.100	−5.07	.004
2nd Group	−1.25	.054	.022	−55.90	.000
3rd Group	−1.36	.081	.033	−41.00	.000

df = 5.

Osteoclast apoptosis has been considered to be a major mechanism of action for the inhibition of bone resorption by strontium in addition to the reduction in cell numbers.

At the end of the third week, the rate of the tooth movements in the experimental sides was reduced significantly, which was explained by a significant increase in the number of osteoblasts with a concomitant significant reduction in the number of osteoclasts. This finding suggested that differences in the appearance of multinuclear osteoclasts could affect the speed of tooth movement and was in accordance with the results of Nishimura et al. (2008).

5. Experiment 2

The difference in the rate of the tooth movement at the end of the first week was found to be statistically insignificant, as had been previously shown by Adachi et al. (1994). This finding was perhaps a result of an initial relapse movement within the first week, which may result from the rebound of the compressed periodontal ligament described by Reitan (1967). Number of osteoblasts in the pressure sides of the experimental molars was significantly increased, while there were no significant differences between the number of osteoclasts in the pressure sides of the experimental and control molars. This finding may be explained by the fact that, at the time of appliance removal, the cellular response may have already been initiated in favor of increasing the number of osteoblasts in the tension side of the molar, which becomes the pressure side after appliance removal (Krishnan and Davidovitch, 2006). Wise and King (2008) showed the biological response to mechanical stimulus applied to a tooth, in which there is an increase in the number of osteoblasts in the tension side and osteoclasts in the pressure side.

At the end of the second week, the rate of the relapse tooth movement was reduced significantly on the experimental side and the number of osteoblasts was significantly increased in

Table 11 Descriptive information on osteoclast numbers in the three groups.

Groups	Variables	Min.	Max.	Mean	SD	SE
1st Group	O.C R side	.90	1.30	1.10	.158	.070
	O.C L side	.69	1.04	.86	.152	.068
2nd Group	O.C R side	1.90	2.30	2.10	.158	.070
	O.C L side	1.75	2.10	1.91	.143	.064
3rd Group	O.C R side	2.80	3.20	3.00	.158	.070
	O.C L side	2.00	2.30	2.16	.151	.067

$N = 6$, O.C = osteoclast number, R = right, L = left.

Table 12 Experimental – control difference of the osteoclast number and the *t*-value with its degrees of freedom and *p*-value.

Groups	Mean $_{difference}$	SD $_{difference}$	SE $_{difference}$	*t* value	*p* value
1st Group	.24	.236	.105	2.27	.085
2nd Group	.19	.235	.105	1.80	.146
3rd Group	.84	.054	.024	34.29	.000

df = 5.

the pressure sides of the experimental molars. As in experiment 1, there were insignificant differences in the osteoclast number between the control and experimental sides despite the retardation of the rate of relapsed tooth movement on the experimental side. This may be explained by the same hypothesis regarding the findings in the second group of experiment 1. By the end of the third week, the rate of the tooth movement on the experimental sides reduced significantly, which was explained by a significant increase in the number of osteoblasts with a significant reduction in the number of osteoclasts. This finding supports the conclusions of Marie et al. (2001) regarding of the dual action of strontium in both increasing osteoblast numbers and reducing osteoclast numbers.

In both experiments, the inhibitory effect of strontium on tooth movement may result in part from the increase in the number of osteoblasts and the reduction in osteoclast numbers in the periodontal tissues subjected to mechanical stress. This finding is inconsistent with the results of many previous studies that demonstrated the positive effects of strontium on bone metabolism by promoting bone formation and decreasing bone resorption (Sila-Asna et al., 2007; Cannata-Andía et al., 2010). We suggest that further histological, immunohistochemical and biochemical investigations are required to enable definitive conclusions to be made regarding the exact mechanism of action of strontium in tissues subjected to experimental tooth movement.

6. Conclusions

The localized injection of strontium has neither adverse effects on the general health of the animal, nor at the injection sites. For the first time, this study demonstrated that the local injection of strontium has an inhibitory effect on experimentally induced and relapsing tooth movement in rats. The local injection of strontium is a promising approach to enhance both orthodontic anchorage and retention.

7. Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgments

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