ORIGINAL ARTICLE

The Synergistic effect of Vitamine D and Prostaglandin E2 On Orthodontic Tooth Movement in Rats

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Background and aim: Orthodontic tooth movement requires remodeling of periodontal tissues, especially alveolar bone. A major objective of investigators is to achieve maximum tooth movement with minimal root damage. The aim of this study is to determine if the rate and amount of orthodontic tooth movement in a sample of rats could be enhanced by the injection of a vitamin D metabolite and Prostaglandin E2.

Materials and Methods: Thirty-Two male Wistar rats were randomly divided into 4 groups of eight rats each:

1- 1.5 IU/ml vitamin D was injected into the thigh muscle after installation the orthodontic appliance in first group (Vit D group)

2- 0.1 ml of 1 mg/ml PGE2 was injected submucosally in the second group (PGE2 group).

3- In the third group, 1.5 IU/ml vitamin D and 0.1 ml of 1 mg/ml PGE2 was injected submucosally (Vit D+ PGE2).

4- Distilled water (0.1 ml) was used in control group .In order to calculate the tooth movement; the distance between the upper right first and second molars was measured.

Results: All groups have a significant difference with control group ($p<0.05$). The most mean OTM was observed in the Vit D+PGE2 group (Mean = 0.702± 0.04897mm) that was significantly higher than the Vit D, PGE2 and control groups ($P < 0.05$).A significant difference ($P< 0.05$) in root resorption was observed between the

PGE2 (0.0192 ±0.000675 mm2) and the other groups.

Conclusion: In order to achieve a decrease in root resorption and an increase in OTM, the combination Vit D+PGE2 is useful and there is a synergism effect with Vit D and PGE2.

Keywords: Prostaglandin E2, Root Resorption, Tooth Movement, Vit D.

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Background:

Orthodontic tooth movement requires remodeling of periodontal tissues, especially alveolar bone. [1-3] 1,25-$(OH)_2D_3$, the active form of vitamin D_3, is known to be a potent stimulator of osteoclastic bone resorption.[4] Vitamin D and its active metabolite, 1, 25,2(OH)D3, together with parathyroid hormone (PTH) and calcitonin, regulate the amount of calcium and phosphorus levels.[5] Vitamin D receptors have been demonstrated not only in osteoblasts but also in osteoclast precursors and in active osteoclasts.[6] intraligamentary injections of vitamin D metabolite, 1,25-dihydroxy cholecalciferol, enhanced the rate of tooth movement in rats due to the well-balanced bone turnover induced by vitamin D.[4,6] Stimulatory action of vitamin D on osteoblasts can help stabilize orthodontic tooth movement.[7] Vitamin D increased bone formation on the pressure side of the periodontal ligament after application of orthodontic forces. [6.7]Local application of vitamin D could intensify the re-establishment of supporting alveolar bone, after orthodontic treatment.[8-10]

One of the factors that is being investigated its effect on tooth movement is prostaglandins (PGs) . [11-13]PGs, especially PGE_2, are potent multifunctional regulators of bone metabolism .[14] PGE_2 induce morphologic changes in osteoclasts and osteoblasts via increased intracellular levels of cAMP [12,14] and exogenous PGE_2 increases the mRNA synthesis and protein secretion of the Receptor Activator of Nuclear factor kappa-B Ligand (RANKL) .[15] However, there was an increase in the amount of root resorption with increasing numbers and concentrations of the PGE_2 injections. [16-18]
S0, reducing the length of treatment may thus help satisfy patients' demands and even lessen the long term sequelae .[19] Increasing mechanical force to reduce the treatment time, a major problem in orthodontic practice, leads to several sequelae and one common major complication of orthodontic treatment has been apical root resorption .[1,20] Therefore, maximum tooth movement with minimal root damage is a major objective of investigators.[21 ,22]

So far no research has been undertaken on injection of vitamin D_3 with PGE2 during orthodontic treatment and its effect on root resorption or tooth movement. The aim of the present study was to compare and investigate the effect of vitamin D with PGE2, on orthodontic tooth movement and root resorption in rats.

MATERIALS AND METHODS

Animals

The experimental protocol was approved by the Institutional Review Board (IRB).
Thirty-two male Wistar rats (6-8 weeks old 230-300 grams weight) were randomly divided into 4 groups of eight rats each. They were fed on NIH-36 diet for mice and rats, with a minimum of 1.15 per cent calcium content.
Fresh drinking water was provided every day and they were cared for according to the Animal Welfare Regulations. Both quadrants of the upper jaws of the first group of animals were used. Therefore this group comprised two groups: control and normal. Eight left the first molar teeth of these eight animals were not placed under orthodontic force. They represented the normal group and were studied for root resorption only. After insertion of an orthodontic appliance on the right side of the upper jaw, distilled water (0.1 ml) was injected at the mesiobuccal mucosa of the right first molars of the control animals. In this way the left side of the upper jaw, which was under no force or injection, was considered the Normal group and the right side of the upper jaw served as the Control.

Appliance design

The orthodontic appliance comprised of a 5 mm long NiTi closed coil spring connected posteriorly to the right first molar and anteriorly to the upper right incisor by a ligature wire and a force of 60 g was applied (Figure 1). Composite bonding material served to fix the ligature wires to the teeth. Orthodontic tooth movement (OTM) was measured with a fealer gauge with an accuracy of 0.01 mm.

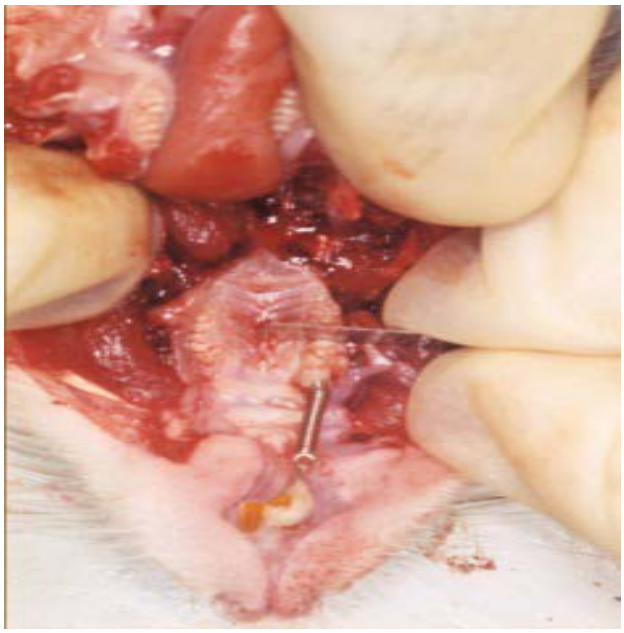


Fig.1. Experimental appliance. An active coiled spring exerted a force of approximately 60 g in the mesial direction

Experimental groups

The animals were divided into following experimental groups:

1- 1.5 IU/ml vitamin D was injected into the thigh muscle after installation the orthodontic appliance in first group (Vit D group)

2- 0.1 ml of 1 mg/ml PGE2 was injected submucosally in the second group (PGE2 group).

3- In the third group, 1.5 IU/ml vitamin D was injected into the thigh muscle and 0.1 ml of 1 mg/ml PGE2 was injected submucosally (Vit D+ PGE2).

4- Distilled water (0.1 ml) was used in control group .In order to calculate the tooth movement. The distance between the upper right first and second molars was measured.

The injections were administered on days 0 and 7.

Histologic evaluation

The animals were sacrificed after 21days using vaporized halothane.The right and left jaw halves of the first eight animals and the right jaw halves of the remnant groups were removed experimental period. The specimens were decalcified by formic acid and placed in paraffin blocks. Sections 5 um thick were obtained at distances of 20 um from the beginning to the end of the root surface. The sections were taken in a mesiodistal direction, going as deep as the middle part of the mesial root of the first molar. Ten to 15 sections of each mesial root were selected, images were taken under a microscope, and resorbed areas on the mesial surface of mesial root of first molar were assessed using computer software. Two examiners recorded the dimensions and the area of the resorbed surface cavities on the mesial surface of these roots.

Statistical analysis

Descriptive statistics (mean, standard error) for each parameter were calculated for all groups. Tooth movement and root resorption score were analyzed by one-way ANOVA and Student-Newman-Keuls test.The SPSS software was used and the significance level set at $p<0.05$.

Results

OTM

Table 1 and figure 2 illustrate the values obtained for OTM in the four groups with an orthodontic appliance. As the F-test in ANOVA demonstrated a significant difference among the four groups, a Student's t-test was used to compare the groups in pairs. The mean OTM in the Vit D +PGE_2 group (Mean = 0.702 ± 0.04897 mm) was significantly higher than the Vit D, PGE_2 and control groups ($P < 0.05$).

Group	Mean	Standard deviation	Range
Vit D	0.395	0.1302	0.33-0.59
PGE_2	0.4700	0.090	0.21-0.90
Vit D+ PGE_2	0.702	0.04897	0.29-0.85
Control	0.2313	0.06643	0.14-0.34

Table 1: Mean and Standard deviation of Orthodontic tooth movement (mm) in the seven experimental groups.

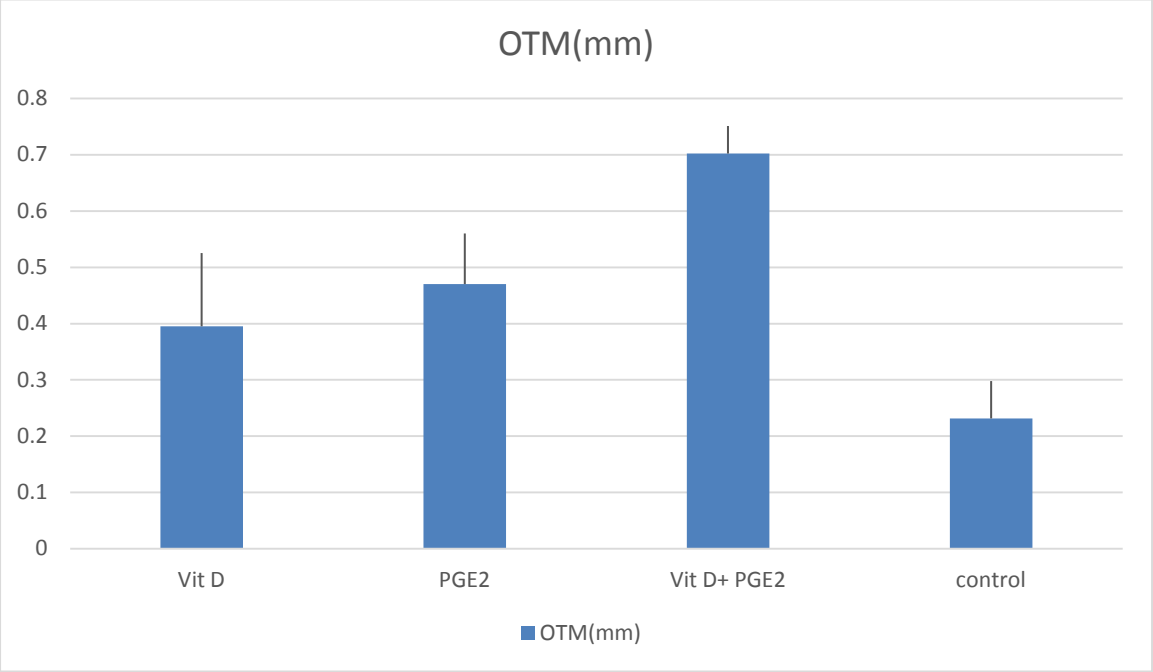


Figure 2: Orthodontic tooth movement (mm) in the Vit D, PGE_2, Vit D+ PGE_2 and control groups. The Vit D, Vit D+PGE_2 and PGE_2 groups had significantly increased OTM, compared with the control group. ($p<0.05$)

Root resorption

Table 2 and Figure 3 illustrate the values obtained for root resorption in the four groups studied. Since there was a variance difference in the four groups, with a *P* value close to 0.05 and the data did not follow a normal distribution curve, a Kruskal-Wallis test was used to confirm the presence of a significant difference in root resorption among the groups. Multiple range tests were then used to compare groups in pairs, which showed a significant difference between the PGE2 and Vit D, Vit D+PGE2 and control groups. No significant difference in root resorption was observed between the other groups.

Table 2: Root resorption (mm^2) in the experimental and control groups.

Group	Mean	Standard deviation	Range
Vit D	0.0020	0.001342	0.001-0.0024
PGE_2	0.0192	0.00198	0.01-0.021
Vit D+ PGE_2	0.00210	0.000675	0.0012-0.0035
Control	0.0024	0.001325	0.0016-0.0026

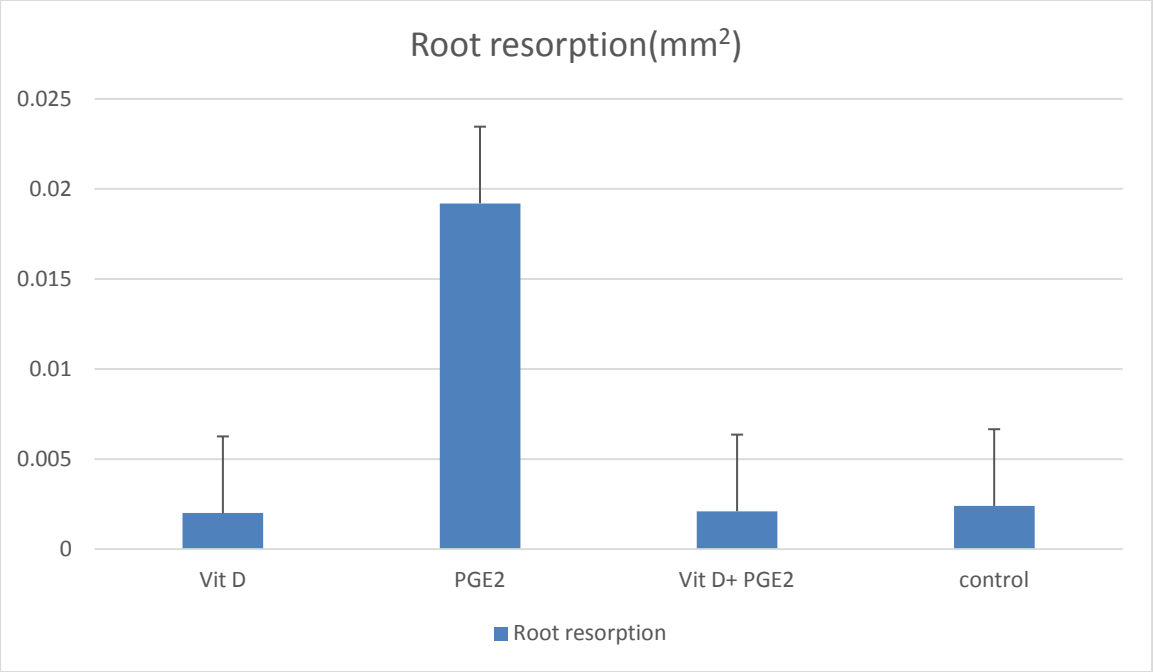
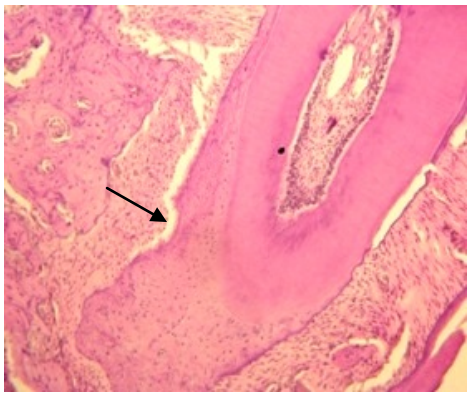


Figure 3: Root resorption (mm^2) in the Vit D, PGE_2, Vit D+ PGE_2 and control groups .There was a significant difference between the PGE2 and Vit D,Vit D+ PGE2 and control groups. No significant difference in root resorption was observed between the other groups. ($p<0.05$)



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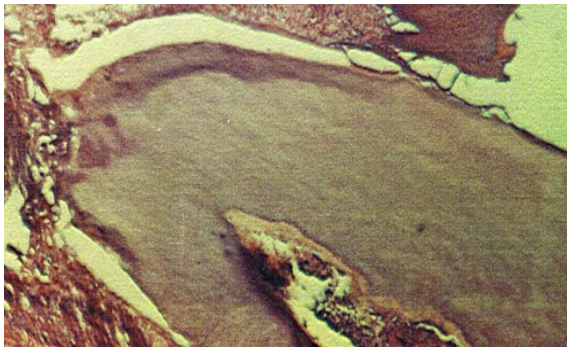

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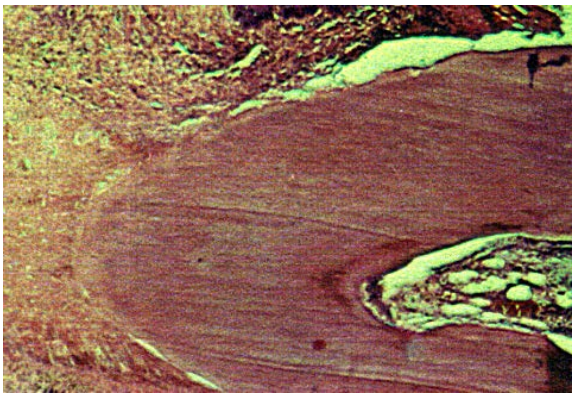

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Figure 4: Histological section of the root of a sample in the Vit D (A), Vit D+ PGE_2 (B) and control groups, receiving saline injection and undergoing orthodontic movement, magnification x25. In PGE_2 group (B), Arrow indicates a large resorptive lacuna.

Discussion

The main findings of the current study are as follows**:**

1)The present results suggest that the Vit D -treated animals showed significantly more tooth movement than the control group but the amount of root resorption was similar to control group. These data corroborate the findings of Collins and Sinclair [6] demonstrated that intraligamentary injections of vitamin D metabolite, 1,25-dihydroxy cholecalciferol, caused increase in the number of osteoclasts and amount of tooth movement during canine retraction with light forces. In 2004 , Kale [9] determined the rate and amount of orthodontic tooth movement in a sample of cats by injection of a vitamin D metabolite 1,25-dihydroxycholecalciferol (1,25D) into the periodontal ligament and observed after 21 days of canine retraction with a light-wire retraction spring, the teeth that had received weekly intraligamentous injections of a solution of 1,25D in dimethylsulfoxide (DMSO) had moved 60% further than matched control teeth ($P < 0.05$). At the histologic level, increased numbers of mononuclear osteoclasts were recruited and activated, resulting in greater amounts of alveolar bone resorption on the pressure side of the periodontal ligament and no obvious clinical, microscopic, or biochemical side effects were noted.These finding were similar to our results.

2) OTM in the PGE2 group in this study occurred significantly faster compared with the control group, which is in agreement with the findings of Yamasaki *et al*,[18,23] Kohoe *et al.* [24] and Boekenoogen *et al.* [17] The reason for the increase might be the bone resorptive effect of PGs after orthodontic loading. Following periodontal injury due to loading, PG is synthesized and PGE2 increases the mRNA synthesis and protein secretion of the receptor activator of nuclear factor kappa-B ligand (RANKL) [16,17] then osteoclastic activity commences, which leads to bone resorption and tooth movement [18] Thus adding PGE to a live environment may induce bone resorption [18].

3) The present results showed that the highest amount of OTM occurred in Vit D+PGE2 group that was significantly higher than Vit D and PGE2 group. No information is available regarding the combination injection of Vit D+PGE2 during OTM. As mentioned before, exogenous PGE2 increases the mRNA synthesis and protein secretion of the receptor activator of nuclear factor kappa-B ligand (RANKL) [16-18] in osteoblasts .Vit D metabolite, 1,25-dihydroxy cholecalciferol, caused increase in the number of osteoclasts. Thus, we can conclude the amount of OTM caused significantly increased due to the synergistic effect between PGE2 and Vit D.

In 1992, Schwartz [25] showed that production of PGE2 in chondrocytes was regulated by vitamin D3 metabolites .In 2013, Liu [26] indicated that Vit D could modulate the synthesis and degradation PGE2 in human lung fibroblasts. So, we suggested further studies on the possible regulation of prostaglandin production and degradation by vit D in osteoblast cells are necessary.

4) The rise in root resorption was significant in the PGE2 group compared with the other groups (Figure 4-A).

5) The present results suggest that the in Vit D + PGE2 animals showed significantly less root resorptive lesions than the PGE2 group. These data suggested that administration of Vit D might provide a protective role on the root surface during OTM, and in those patients that present spontaneous root resorption lesions. In 2004, Kawakami[4] showed that the local application of 1, 25(OH) 2D3 enhances the reestablishment of supporting tissue, especially alveolar bone of teeth, after orthodontic treatment. Perhaps the effect of vitamin D on the supporting structures may explain the protective effect on root structure in our study.

Conclusion:

Thus, the results of the present study indicate that in order to achieve a decrease in root resorption and an increase in OTM, the combination Vit D+PGE2 is useful and there is a synergism effect with Vit D and PGE2. Using an accurate and appropriate combination of local and systemic factors, it might be possible to reduce treatment duration with fewer complications following orthodontic treatment.

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