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The effect of prostaglandin E_2 and calcium gluconate on orthodontic tooth movement and root resorption in rats

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SUMMARY Possible modifications in orthodontic tooth movement (OTM) and root resorption as a result of local injections of prostaglandin E_2 (PGE_2) alone and with calcium gluconate (Ca) formed the aim of the present study. Twenty-four 8-week-old male Wistar rats were selected and randomly divided into three groups of eight. Both quadrants of the upper jaws of the first group of animals were used; therefore this group comprised two groups: control and normal. The upper left first molars of these eight animals were not placed under orthodontic force and received no injection, to serve as the normal group, considered for root resorption comparison only. The control group had localized submucosal injections of normal saline on the buccal side of the upper right first molar. In the third group, 0.1 ml of 1 mg/ml PGE_2 was injected at the same site and the fourth group received an intraperitoneal injection of 200 mg/kg Ca (10%) in addition to the PGE_2. All the injections were performed on days 0 and 7. The orthodontic appliance consisted of a closed coil spring ligated to the upper right first molar and incisor, exerting a force of 60 g during the 21-day experimental period, after which the animals were sacrificed. Palatal halves were removed for histological examination and for calculation of the amount of root resorption.

Statistical analysis of data showed a significant ($P < 0.05$) acceleration in OTM after PGE_2 injection compared with the control group. The addition of Ca reduced OTM but a significant increase ($P < 0.05$) was still recorded. A significant difference ($P < 0.05$) in root resorption was only observed between the PGE_2 and normal groups. The findings show the importance of calcium ions working in association with PGE_2 in stabilizing root resorption while significantly increasing OTM.

Introduction

Fixed orthodontic appliances compromise both aesthetics (Proffit and Fields, 2000) and oral hygiene (Al-Khateeb *et al.*, 1998). Reducing the length of treatment may thus help satisfy patients' demands and even lessen the long-term sequelae. An injection of biochemical agents such as prostaglandin (PG) is one method that has proven effective and significantly increased tooth movement (Yamasaki, 1983; Yamasaki *et al.*, 1980, 1982; Chao and Shih, 1988; Kalange, 1988; Lee, 1990; Brudvik and Rygh, 1991; Leiker, 1993). The mechanism of action of prostaglandin E_2 (PGE_2) can be explained by the pressure–tension theory of tooth movement, which assumes chemical signals to be cell stimulants that lead to tooth movement (Rygh *et al.*, 1986; Rygh, 1989). According to this theory pressure causes changes in the periodontal ligament (PDL) blood circulation and the resultant release of chemical mediators. Inflammatory mediators may act in concert and produce synergistic potentiation of prostanoid formation in cells of the human PDL (Ransjö *et al.*, 1998). There is evidence that PG is released when cells are mechanically deformed (Rodan *et al.*, 1989). Furthermore, PGE_2 plays an important role as a mediator of bone remodelling under mechanical forces (Yamasaki *et al.*, 1980, 1982; Chao and Shih, 1988; Lee, 1990). PGE_1 is not produced in significant quantities in humans *in vivo* (Campbell and Halushka, 1996).

One common major complication of orthodontic treatment has been apical root resorption (Brezniak and Wasserstein, 1993a,b; Killiany, 1999). Its pathogenesis has been assumed to be the removal of necrotic tissue from areas of the PDL that have been compressed by an orthodontic load. It is believed that PGs are involved in root resorption (Harris *et al.*, 1973). In addition, various factors influence the amount of root resorption, including the proven effect of systemic calcium (Roberts, 2000). Low levels of calcium cause secondary hypoparathyroidism and an increase in secretion of parathyroid hormone (PTH) and vitamin D active metabolites. PDL tissue is involved in the formation of root resorptive cells and root resorption (Shiraishi *et al.*, 2001). The number of osteoclasts and their progenitors has been shown to rise in rat PDL following PTH interference (Soma *et al.*, 1999). Osteoclast-like cells can cause root as well as bone resorption under normal treatment conditions (Reitan and Rygh, 1994).

Drugs may be used in future to facilitate or inhibit tooth movement during orthodontic treatment (Proffit and Fields, 2000). Whilst PGE_1 has been clinically applied

to increase the rate of tooth movement (Yamasaki *et al.*, 1984), so far no research has been undertaken on injection of calcium compounds during orthodontic treatment and its effect on root resorption or tooth movement. The aims of this study were to apply PGE_2 and calcium gluconate (Ca) to increase tooth movement and limit root resorption (subsequent to parathyroid gland suppression).

Materials and methods

Twenty-four 8-week-old male Wistar rats weighing 230–300 grams were randomly divided into three groups of eight. 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 molar teeth of these eight animals were not placed under orthodontic force. They represented the normal group and were studied for root resorption only. Distilled water (0.1 ml) was injected at the mesiobuccal mucosa of the right first molars of the same animals after insertion of an orthodontic appliance on the right side of the upper jaw. 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. In addition to orthodontic force, 0.1 ml of 1 mg/ml PGE_2 dissolved in 1 per cent lidocaine was injected submucosally at a similar site for the eight animals in the third group. In the fourth group, PGE_2 was injected submucosally and 10 per cent Ca (200 mg/kg) was injected intra-peritoneally (Marcus, 1996). The injections were administered on days 0 and 7. The orthodontic appliance comprised a 5 mm long closed coil spring connected posteriorly to the right first molar and anteriorly to the upper right incisor by a ligature wire. A force of 60 g was applied. Composite bonding material served to fix the ligature wires to the teeth. Orthodontic tooth movement (OTM) was measured with a gauge with an accuracy of 0.01 mm.

The animals were sacrificed using vaporized halothane. The right and left jaw halves of the first eight animals and the right jaw halves of the third and fourth groups were removed after the 21-day experimental period. The specimens were decalcified by formic acid and placed in paraffin blocks. Sections 5 μm thick were obtained at distances of 20 μm 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.

Results

OTM

Table 1 illustrates the values obtained for OTM in the three groups with an orthodontic appliance. As the F-test in ANOVA demonstrated a significant difference among the three groups, a Student's *t*-test was used to compare the groups in pairs. The mean OTM in the PGE_2 ($P = 0.0396$) and $PGE_2 + Ca$ ($P = 0.0024$) groups was significantly higher than in the control group, although the $PGE_2 + Ca$ group demonstrated a non-significant decrease ($P > 0.05$) in OTM, in comparison with the PGE_2 group. No other significant differences were found (Figure 1).

Root resorption

Table 2 illustrates 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 amongst the groups. Multiple range tests were then used to compare groups in pairs, which showed only a significant difference between the PGE_2 and normal groups (Figure 2).

Table 1 Orthodontic tooth movement (mm) in the control, PGE_2, and PGE_2 + Ca groups.

	Control	PGE_2	PGE_2 + Ca
Mean	0.2162	0.4700	0.4012
Standard deviation	0.0995	0.2799	0.1007
Range	0.14–0.45	0.20–0.91	0.29–0.57

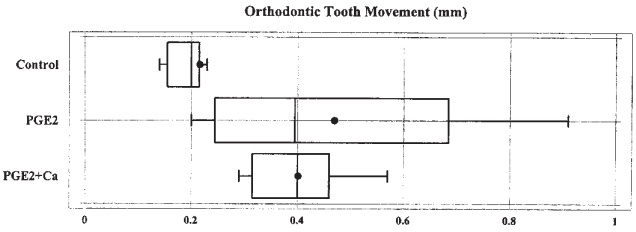

Figure 1 Orthodontic tooth movement (mm) in the control, PGE_2, and $PGE_2 + Ca$ groups. The last two groups had significantly increased OTM, compared with the control group. The rectangle extends from the lower to the upper quartile. The centreline of each box shows the median and the solid circle indicates the mean. The whiskers extend from the minimum to the maximum values.

Table 2 Root resorption (mm^2) in the normal, control, PGE_2, and PGE_2 + Ca groups.

	Normal	Control	PGE_2	PGE_2 + Ca
Mean	0.0026	0.0081	0.0192	0.0113
Standard deviation	0.0018	0.0043	0.0198	0.0111
Range	0.0004–0.0063	0.0020–0.0136	0.0014–0.0634	0.0015–0.0342

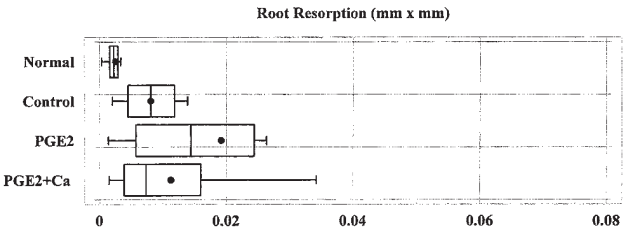

Figure 2 Root resorption (mm^2) in the normal, control, PGE_2, and PGE_2 + Ca groups. The only significant difference was between the PGE_2 and normal group. The rectangle extends from the lower to the upper quartile. The centreline of each box shows the median and the solid circle indicates the mean. The whiskers extend from the minimum to the maximum values.

Discussion

Different methods have been utilized to increase tooth movement, such as modifying force magnitude (Storey and Smith, 1952; Furstman *et al.*, 1971), vitamin D metabolite injection (Takano-Yamamoto *et al.*, 1992), steroid therapy (Ong *et al.*, 2000), altering bone metabolism by PTH (Soma *et al.*, 1999) and thyroxin intervention (Shirazi *et al.*, 1999). PGE has already shown promise in accelerating OTM and is known as a movement mediator. Despite improvements in the understanding of the role of cAMP (Kent *et al.*, 1980; Yamasaki, 1983), calcium (Nakago-Matsuo *et al.*, 1996), collagenase (Dietrich *et al.*, 1975), cytokines (Saito *et al.*, 1991; Grieve *et al.*, 1994; Ransjö *et al.*, 1998), and PGs (Saito *et al.*, 1991; Grieve *et al.*, 1994), the exact mechanism of how orthodontic force turns into cellular response remains unknown (Engström *et al.*, 1988).

OTM in the PGE_2 group in this study occurred significantly faster compared with the control group, which is in agreement with the findings of Yamasaki *et al.* (1980, 1982, 1984), Kohoe *et al.* (1996) and Leiker (1993). 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 osteoclastic activity commences, which leads to bone resorption and tooth movement (Yamasaki, 1989). Thus adding PGE to a live environment may induce bone resorption (Yamasaki, 1983).

The combined injection of PGE_2 and Ca reduced OTM but despite this decrease it still occurred at a significantly increased rate compared with the control group. No information is available regarding injection of calcium compounds during OTM. Goldie and King (1984) found that systemic calcium deficiency increased OTM. Midgett *et al.* (1981) demonstrated significantly decreased bone density and increased bone remodelling in animals with hyperparathyroidism, indicating that the reduction in bone density seems to facilitate tooth movement within bone. It can be inferred from the above that the hypoparathyroidism caused by calcium injection in the present study should have inhibited bone remodelling and resisted tooth movement whereas this was not the case. This can be explained by the dominant role of PGE_2 with a dose of 1 mg/ml, although a minor insignificant drop was observed in OTM.

There have also been attempts to minimize root resorption (Poumpros *et al.*, 1994; Shirazi *et al.*, 1999). Steroids have been applied successfully for this purpose (Ong *et al.*, 2000). The mechanism of root resorption is probably the action of macrophages, which eliminate the hyalinized zone of the PDL by secreting PG after orthodontic loading (Moyers, 1988). Systemic factors may be involved in the regulation of tissue degradation (Aubauch *et al.*, 1981). Reports of patients at high risk of developing root resorption suggest the impact of factors other than force (Hollender *et al.*, 1980; McFadden *et al.*, 1989) and although not definitely proven, a close correlation has been observed between root resorption and hypothyroidism (Newman, 1975). As previously stated, low levels of serum calcium can also evoke bone and root resorption. A change in serum calcium level is a determining factor for root resorption despite the decisive role of PTH in regulation of bone resorption (Engström *et al.*, 1988). It thus seems likely that raised serum calcium levels may inhibit PTH secretion and therefore inhibit root resorption.

Statistical analysis indicated no significant difference in root resorption between the normal and control groups (Figure 3), which is contrary to the findings of Boekenoogen *et al.* (1996). The shorter experimental period and a difference in the types of injection plus the number of surfaces considered for measurement may explain the difference.

Root resorption showed an increased trend in the PGE_2 group (Figure 4) compared with the control group, although this was not significant. Leiker (1993) found opposite results, perhaps because he measured the whole mesial surface for resorption. Kalange (1998)

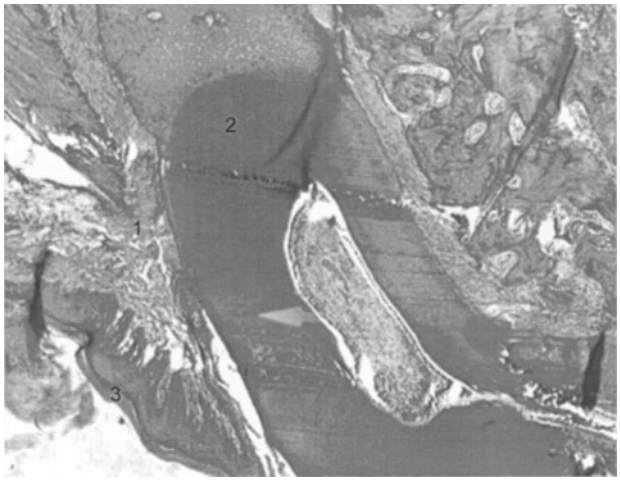

Figure 3 Histological section of the root of a sample in the control group, receiving saline injection and undergoing orthodontic movement, magnification ×25. Arrow indicates a resorption area. 1, bone; 2, root; 3, epithelium.

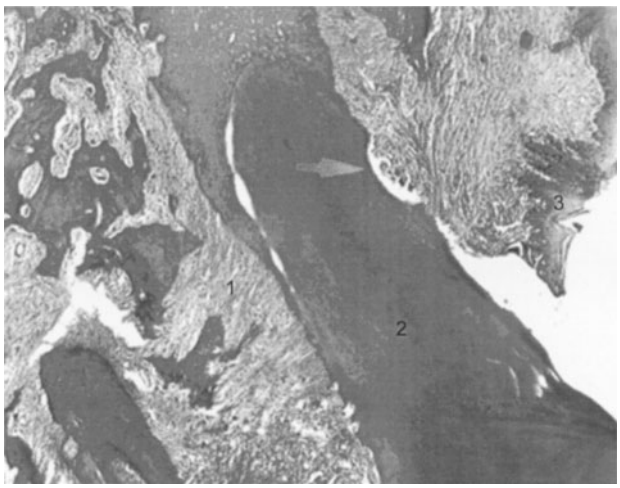

Figure 4 Histological section of the root of a sample in the PGE_2 group, receiving prostaglandin E_2 injection and undergoing orthodontic movement, magnification ×25. Arrow indicates a large resorptive lacuna. 1, bone; 2, root; 3, epithelium.

and Brudvik and Rygh (1991) reported results similar to those found in the present study, despite shorter experimental periods. Boekenoogen *et al.* (1996), who also carried out an extensive investigation using various dosages and intervals, came to the same conclusion.

The rise in root resorption was significant in the PGE_2 group compared with the normal group, which was not surprising in view of the destructive effect of PGE_2 in cysts in the oral region.

No significant differences were found for root resorption in the PGE_2 + Ca group (Figure 5) compared with either the normal or control group. Goldie and King (1984) reported a reduction in root resorption for calcium deficient rats; however, Bielaczyc and

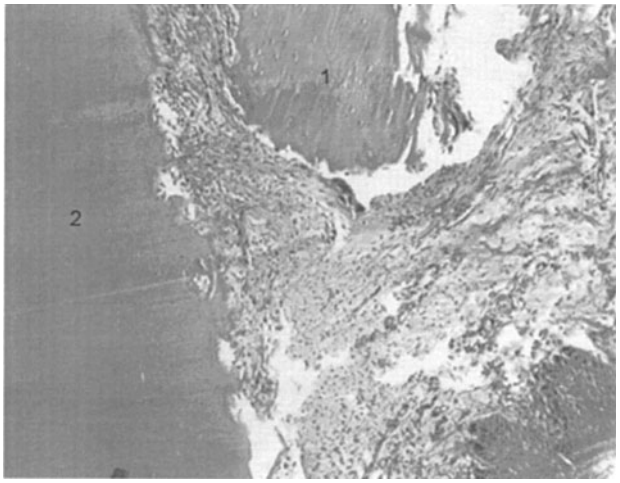

Figure 5 Histological section of the root of a sample in the PGE_2 + Ca group, receiving prostaglandin E_2 and calcium injections and undergoing orthodontic movement, magnification ×100. Small resorptive lacunae and some giant cells are apparent. 1, bone; 2, root; 3, epithelium.

Golebiewska (1997) demonstrated a rise in root resorption with a diet low in calcium and deficient in vitamin D. The tendency towards a reduction of resorption in the PGE_2 + Ca group may be a result of the transient hypoparathyroidism and diminished resorptive activity subsequent to injection of the calcium compound.

Conclusion

In light of the trend toward a decrease in root resorption and an increase in OTM in the PGE_2 + Ca group, further investigations are required with different doses and time periods. 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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