Original Article

The Effect of Celecoxib on Orthodontic Tooth Movement and Root Resorption in Rat

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Abstract

Objective: Inhibition of prostaglandin (PGs) production leads to decrease in orthodontic tooth movement (OTM). It is not known whether inhibition of cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2) is the key mechanism for this effect. In this study, the effect of celecoxib, a highly-selective COX-2 inhibitor, was investigated on OTM in rats.

Materials and Methods: Four groups of male rats (seven animals in each goup) were used in the study. A 5mm-long nickel-titanium closed-coil spring was ligated between the right maxillary incisor and the first molar of each rat to deliver an initial force of 60g. All four groups recieved orthodontic appliances, group 1 received no injections, group 2 received celecoxib injections (0.3 mg in 0.1 ml saline solution), group 3 recieved normal saline injections (0.1 ml saline solution), and group 4 recieved needle penetration without injecting any solution. The local injections were carried out every 3 days for 18 days. All injections were subperiosteal and given in the upper right first molar mucosa. The animals were sacrificed 21 days after appliance insertion and OTM was measured.

Results: In the animals treated with celecoxib a statistically significant decrease in OTM was observed compared with the other groups. Histological findings revealed that osteoclast count was significantly lower in group 2 compared with the other groups ($P<0.05$). The amount of root resorption showed a slight, but nonsignificant decrease in group 3.

Conclusion: This study suggests that celecoxib decreases OTM and osteoclast count. This might be the result of COX-2 enzyme inhibition and subsequent decrease in prostaglandin production.

Key Words: Orthodontics; Tooth Movement; Celecoxib; Prostaglandins; Cyclooxygenase; Rats

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INTRODUCTION

Orthodontic tooth movement (OTM) results from the response of periodontal tissue to orthodontic force [1-3] and is suggested to be the consequence of the contribution of several mediators, a group of which are prostaglandins

(PGs) [4,5]. PGs are produced from membrane phospholipids by the sequential action of phospholipase A2 and cyclooxygenase (COX) [6]. PGE1 and PGE2 have been shown to stimulate bone [7-11] and root resorption [12, 13]. It has also been shown that PGs influence OTM through stimulation of the osteoclastic process of bone resorption [14].

Production of PGs by osteoblastic cells is dependent on the presence of enzyme COX and arachidonic acid in place [15, 16].

Two distinct forms of COX have been identified, a constitutive form (COX-1) that mediates physiological functions, and an inducible form (COX-2), which is usually associated with pathological conditions, such as inflammation, pain and several types of cancers [15, 17-20], so the use of COX inhibitor drugs can impact the rate of tooth movements by preventing or at least reducing PG production [15, 17]. Several studies have been reported that non steroidal anti inflammatory drugs (NSAIDs) such as ibuprofen, acetylsalicylic acid and naproxen sodium can relieve the generated pain by orthodontic appliances, but the extended use of these medicines may also have some adverse effect on tooth movement [17, 21, 22]. Osteoclastic response due to orthodontic force is the prerequisite of tooth movement, but the mechanism of activation for this process is not completely understood [16, 23]. Okada et al. reported that stimulated production of PGs by osteoblasts requires both the induction of COX-2 expression and the availability of arachidonic acid substrate.

They also suggested that a role for COX-2 in bone resorption might be most evident when bone resorption is accelerated [24].

The effect of PGs on root resorption has also been reported in some studies [25]; therefore, the other effects of PG inhibitor drugs may be lessening of root resorption [25].

Celecoxib is a highly selective inhibitor of COX-2 and has been shown to be an anti-inflammatory and analgesic agent with gastrointestinal safety [21, 26].

Igarashi found that bone resorption and osteoclastogenesis may be inhibited by celecoxib and they concluded that COX-2 dependent PGs synthesis is critical for bone resorption and osteoclastogenesis [27].

The effect of coxibs on tooth movement is controversial [17, 18, 28].

Some studies have shown that oral administration of celecoxib, a kind of coxibs, had no effect on orthodontic tooth movement in rats [18].

The aim of this study was to determine the effect of local administration of celecoxib on orthodontic tooth movement and root resorption.

MATHERIALS AND METHODS

Animals and drugs

In this interventional study, twenty eight 5-week male Sprague-Dawley rats with an initial body weight of 250-300 grams (Razi Institute, Tehran, Iran) were used. The animals were acclimatized for one week in plastic cages with a standard 12-hour light-dark cycle and fed a diet of soft laboratory food to minimize any discomfort due to orthodontic appliance insertion and the risk of appliance displacement.

This study conformed to the Guide for the Care and Use of laboratory animals published by US National Institute of health (NIH Publication No 85-23, revised 1985). Celecoxib (Searle, USA) was prepared in saline solution as a suspension.

Orthodontic treatment

The animals were anesthetized with an intraperitoneal injection of ketamine (Rotex medica, Trittau, Germany) (50 mg/kg) and xylazin HCL (Rompoun, Bayer, Leverkusen, Germany) (6 mg/kg). A 5mm nickel-titanium closed-coil spring (Niti, 3M unitek, Monorova, Claif, Hitek 0.006 x 0.022 inch) was ligated between the right maxillary first molar and incisors of each rat to deliver an initial force of 60gr [29] (Fig 1).

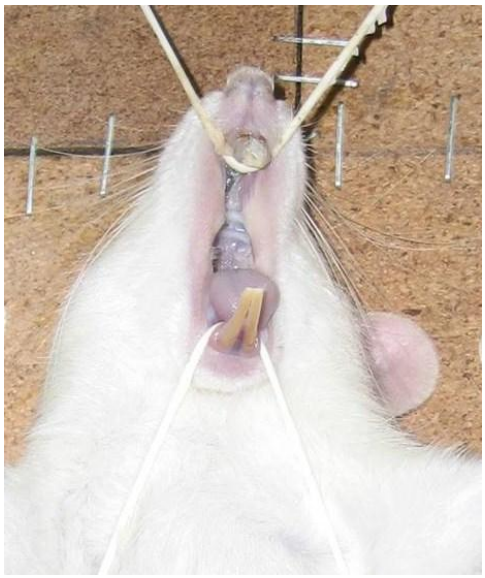

Fig 1. A 5 mm nickel-titanium closed-coil spring was ligated between the right maxillary first molar and incisors of each rat to deliver an initial force of 60 gr

The spring was fixed in place via 0.01 inch ligature wires that surrounded the molar tooth and the incisor.

Due to lack of undercuts in the incisor area, a cervical groove was prepared on the tooth, where the ligature wire was seated.

In order to minimize the distal movement of the incisor and to reinforce anterior anchorage, the right and left incisors were joined with composite resin and acted as a unit. Because of the load-deflection curve of these springs they were activated only once at the beginning of the study during the 3-week period of the experiment.

Measurement of tooth movement

The weight and general condition of all animals and the status of the appliances were evaluated. At the end of the experimental period, the animals were sacrificed with chloroform, and the maxillae were sectioned and prepared for measurement.

Tooth movement was determined by measuring the space created between the right first and second maxillary molars with standard interproximal feeler gauge gauge (Mitutoyo Co., Kawasaki-Shi, Japan) calibrated to 0.01mm increments.

The space was measured before appliance removal to avoid any probability relapse.

Experimental group rats were randomly divided into four groups each containing 7 rats:

1-Control appliance group without injections.

2-Celecoxib group in which celecoxib (0.3 mg in 0.1 ml saline solution, sub-periosteal) was administered.

3-Control saline group in which normal saline injections (0.1 ml saline solution, sub-periosteal) were administered.

4-Needle group only had a needle penetration into the subperiosteal space without injecting any solution that acted as a sham control group

Orthodontic appliances were placed in all groups. All injections were administered as local subperiosteal injections in the buccal mucosa of the upper right first molar at 72-hour intervals starting from the first day of appliance insertion to the 18th day (3 days before the end of the study).

To have a control for OTM, the OTM of the corresponding teeth of the opposite side of the maxilla was measured in each rat.

In order to make the injections possible, the animals were made drowsy by a brief administration of ether before each injection.

Histological study

After measuring tooth movement, block sections of the maxilla were dissected and placed in 10 percent formalin for 10 days for fixation and then decalcified with 5% formic acid for 5 days and embedded in paraffin.

Parasagital sections of the teeth were cut at 6 µm and stained with hematoxylin and eosin by conventional histologic methods.

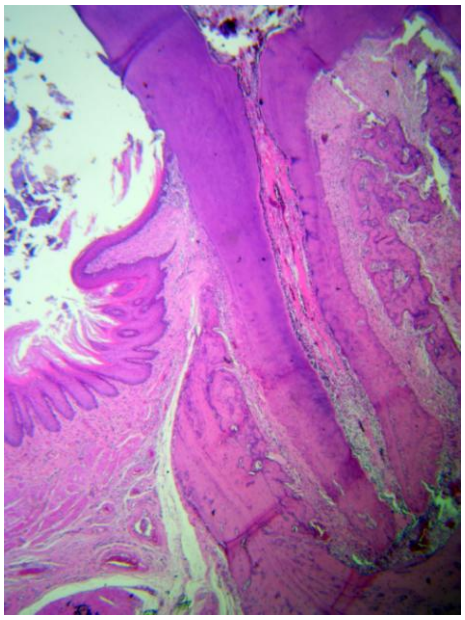

Fig 2. Photomicrograph showing a histological section of the mesial root of the first molar (×40).

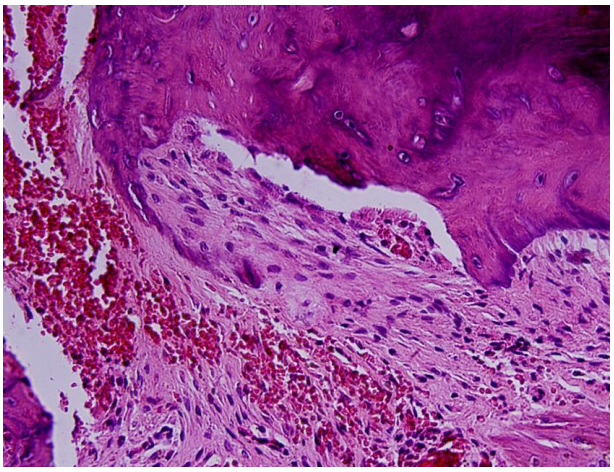

Fig 3. Resorptive space at the cementum. Inflammatory tissue, osteoclast cell and resorbed cementum could be seen (×100).

After preparation of the specimens, 8 sections were achieved from each tooth. Histological analysis of root resorption was performed on the mesial and distal surfaces of the mesial root of the maxillary first molar.

The mesial root was selected for several reasons [12, 13]. First it was by far the largest of the five roots on the maxillary first molars. Second, it was typically the only root that remained intact during dissection of the alveolar bone.

Third, the mesial root, which is located buccolingually in the middle of the tooth, was in approximately the same plane as the applied force. Fourth, this root had been examined in previous studies and therefore could be compared with findings from previous investigations [30].

The maximum depth of each resorption lacunae was measured at the deepest point by using the distance from the bottom of the cavity tangent to the line passing the intact root surfaces from both sides of the cavity by means of a routine light microscope (BX-41, Olympus, Tokyo, Japan) [30] (Fig 2). The mean value for each tooth was achieved by averaging the eight sections and considered as a cavity depth. The number of osteoclasts was counted on the bone surface in all eight sections of each specimen. The histologic criteria for osteoclasts were multinuclear and eosinophylic cells on the bone surface [5] (Fig 3).

Each case was re-counted twice and the mean value was selected as the osteoclast count for each case.

Statistical analysis

Data obtained from OTM, root resorption and osteoclast count was expressed as the mean ± standard error of the mean. One way ANOVA was conducted to determine differences in the amount of tooth movement and root resorption among the groups, and followed by Turkey-test for evaluating the differences between groups when differences were significant. $P< 0.05$ was considered statistically significant.

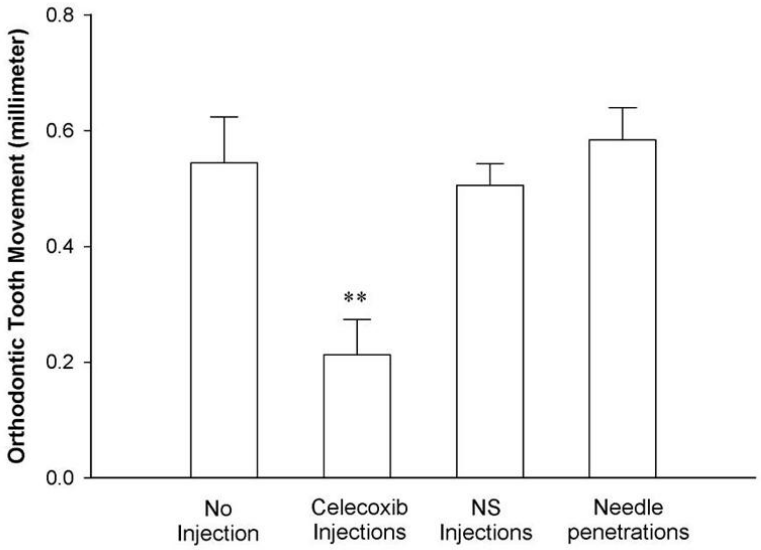

Fig 4. Orthodontic tooth movement (OTM) have been shown in four experimental groups. Group 2 (celecoxib group) showed the least amount of OTM between the groups

RESULTS

OTM changes

All groups showed evidence of tooth movement. No movement was detected in the contra-lateral teeth. The measurements are shown in Fig 4.

Analyzing the OTM data of the four experimental groups with one-way ANOVA showed a significant difference between groups ($F_{3, 24} = 7.917$, $P < 0.01$). Further analysis with Turkey test and post hoc comparisons revealed that the mean OTM in group 2 (0.21 ± 0.06 mm) was significantly lower than group 1, 3, and 4. (0.54 ± 0.08 mm, 0.51 ± 0.04 mm, 0.58 ± 0.06 mm, respectively).

Histological findings

Root resorption was observed in all groups. The amount of resorption at the distal surface of the mesial root was less than the mesial surface of the same root.

But statistical analysis of the amount of root resorption in the four experimental groups did not show a significant difference (One-way ANOVA, $F_{3, 24} = 1.973$, $P > 0.05$) (Fig 5).

Osteoclasts, inflammatory cells and recently formed bone and resorptive areas were observed in all groups. In the celecoxib group, less inflammatory cells and less root and bone resorption were detected. The analysis of the differences between mean osteoclast counts in the four experimental groups using one-way ANOVA revealed a significant difference ($F_{3, 24} = 6.198$, $P<0.01$).

Further analysis with post hoc comparisons of Turkey test showed that in group 2, the mean osteoclast count significantly decreased when compared with the other groups (Fig 6).

DISCUSSION

Activation of orthodontic appliance produces inflammation and remodeling response in the periodontal tissue and alveolar bone that leads to OTM. The involvement of PGs in mediating OTM is well-established [31]. Acceleration of OTM in conjunction with exogenous PG administration has been reported previously [4, 5, 13]. NSAIDs that inhibit PG production have been shown to markedly reduce the speed of OTM [32].

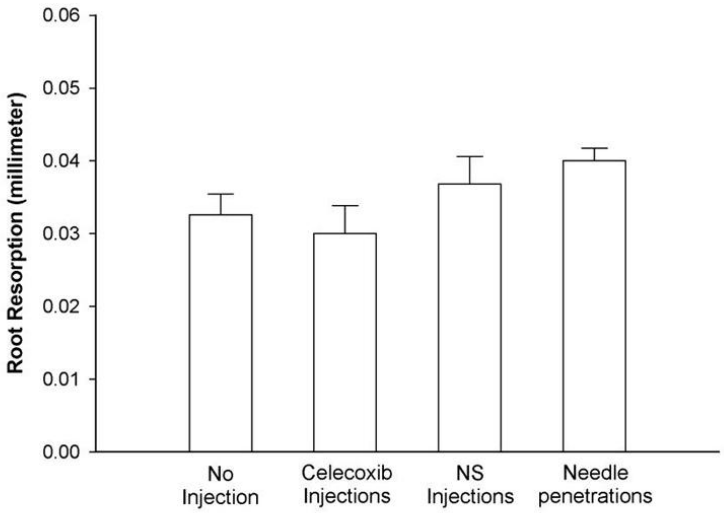

Fig 5. Celecoxib local injection group showed less root resorption than the other groups

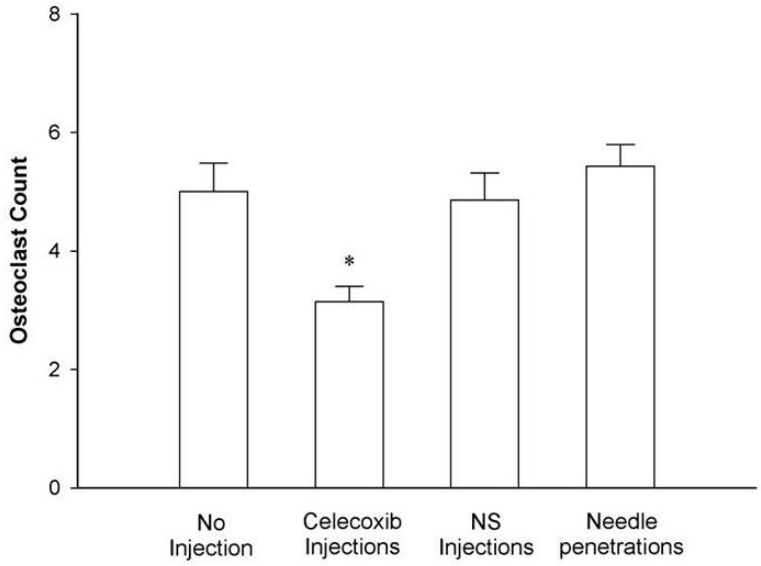

Fig 6. Histological measurement revealed the minimum amount of osteoclast cell number with the local administration of celecoxib

The findings of the present study suggest that PGs produced by COX-2 may play an important role in bone resorption and OTM. OTM in group 2 was significantly decreased in comparison with the other appliance groups. This means that inhibiting COX-2, while preserving COX-1 activity leads to a decrease in bone resorption and consequently in OTM. This is consistent with the findings of a study conducted by Okada et al. [24], who confirmed the major role of COX-2 in bone resorption and suggested that its role was most evident when bone resorption was accelerated. In a study performed by Igarashi et al. [27], they observed that bone resorption would be inhibited by using celecoxib. Hauber Gameiro et al. [17] also showed that celecoxib decreased the rate of OTM. In other studies carried out by de Carlos et al. [15, 16], they reported that rofecoxib, a kind of COX-2 inhibitor, has a negative effect on OTM and reduces orthodontic pain without a significant effect on OTM. Similar results were detected in a recent study by Hashemi et al. that showed celecoxib reduced OTM, but there was no direct relationship between the dose of celecoxib and the decrease of tooth movement [33].

In the other new study by Shaza et al., findings revealed that administration of celecoxib did not reduce bone resorption or interfere with tooth movement in rats.

These results were not consistent with our study, maybe because of the different methodology [34].

In this study, a slight but not significant decrease in root resorption was detected in group 2. This result is consistent with the study of Gameiro et al. [25] who showed that celecoxib has no effect on root resorption. Sekhavat et al. [30] reported the same findings by using misoprostol (PGE1 analog), but others revealed different results, as local injection of PGE2 significantly increased root resorption [12, 13].

Hashemi et al. also observed that using celecoxib especially in high dose decreased the number of resorbtion lacuna on the root surface and therefore had a protection effect on root resorption. [33].

The number of osteoclasts were significantly lower in group 2 compared with other appliance groups. This indicates that the formation of osteoclasts could be inhibited by celecoxib probably by the inhibition of COX-2. This is parallel with our OTM findings as well as the reports by Igarashi [27] who showed that by using celecoxib, osteoclastogenesis could be inhibited. Yamasaki and Miura [5] also reported that indomethacin (a non selective COX inhibitor) inhibited the appearance of osteoclasts.

They also showed that injecting PGE1 and PGE2 in the gingiva resulted in the appearance of osteoclasts.

CONCLUSION

Injection of celecoxib (highly selective COX-2 inhibitor) resulted in a decrease in OTM. A parallel significant decrease in osteoclast count and a slight, but nonsignificant decrease in root resorption were also detected. It may be hypothesized that celecoxib decreases OTM probably by inhibiting COX-2 enzyme and consequent PG production that leads to decreased bone remodeling.

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