Original Article

Effects of Short- and Long-Term Celecoxib on Orthodontic Tooth Movement

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ABSTRACT

Objective: To test the hypothesis that short- and long-term celecoxib administration has no effect on orthodontic tooth movement.

Materials and Methods: Male Wistar rats were submitted to short- (3 days) and long-term (14 days) celecoxib administration, while the respective control groups received equivolumetric saline intraperitoneal injections. The upper left first molars of all rats were moved mesially for 14 days by a fixed orthodontic appliance exerting 50 g force upon insertion. After the experimental period, tooth movement was quantified and tissues around the first molar were processed for tartrate-resistant acid phosphatase (TRAP) histochemistry. The amount of tooth movement and the number of TRAP-positive cells on the alveolar bone surface were evaluated.

Results: The amount of tooth movement was significantly reduced in rats submitted to short- and long-term celecoxib administration, while the number of osteoclasts on the alveolar bone did not differ between the four groups studied.

Conclusions: The hypothesis is rejected. Although celecoxib administration did not affect the number of osteoclasts, the osteoclast activity might be reduced, which could explain the inhibition of tooth movement observed in the celecoxib-treated animals. These results indicate that orthodontists should be aware of patients under short- and long-term therapy with celecoxib.

KEY WORDS: Celecoxib; Tooth movement; Orthodontics

INTRODUCTION

Patients undergoing orthodontic treatment usually experience some degree of pain or discomfort.[1] Surveys performed to determine the experience of orthodontic pain have rated it as a key deterrent to orthodontic therapy and a major reason for discontinuing treatment.[2–5]

The most common group of medications used in orthodontics for pain relief consists of nonsteroidal anti-inflammatory drugs (NSAIDs).[6–8] These drugs function by inhibition of the enzyme cyclooxygenase (COX), which modulates the transformation of prostaglandins (PGs) from arachidonic acid in the cellular plasma membrane.[9] PGs, such as PGE1 and PGE2, are important mediators of bone resorption.[10,11] Two isoforms of COX have been described: the constitutive COX-1 and the inducible COX-2. The COX-1 is considered important in tissue homeostasis and the COX-2 is transcriptionally induced by cytokines and appears to be important in the development of inflammation.[12] Numerous studies evaluated the pain-reducing effects of various NSAIDs, including ibuprofen,[13] acetylsalicylic acid,[14] and naproxen sodium.[15] These studies demonstrated that NSAIDs effectively reduce pain and discomfort caused by the periodic activation of orthodontic appliances, but these drugs may also affect the sequence of tooth movement by inhibiting or at least by

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reducing the associated inflammatory and bone resorptive processes.

One approach to deal with this problem is the use of selective COX-2 inhibitors, also named coxibs, which are replacing conventional NSAIDs, especially for chronic inflammatory conditions.[16,17] It has previously been shown that some coxibs (celecoxib[11,18] and parecoxib[11]) do not interfere in the rate of orthodontic tooth movement. However, the specificity of coxib can account for different effects of these drugs on tooth movement.[11] One example is rofecoxib, a drug that can disturb the process of tooth movement.[11,19] In addition, this drug has been the object of debate and was even withdrawn from the market due to reports of unwanted cardiovascular and renal side effects.[20] Considering that COX-2 is upregulated when orthodontic forces are applied,[11] it is possible that coxibs would interfere in tooth movement.

It is important to point out that the preemptive or preoperative administration of analgesics, in order to decrease postoperative pain, has become the focus of recent research in orthodontics.[15,21,22] Some authors also recommended two postoperative doses, in addition to a preoperative dose, for complete pain control during each orthodontic appointment.[15] It was suggested that low doses administered for 1 or 2 days in the initial stages will not affect the tooth movement process as such.[8] However, this hypothesis was not substantiated by controlled experimental studies.

On this background, the present study was designed to investigate possible effects of short- and long-term celecoxib administration on tooth movement induced by experimental orthodontic force application.

MATERIALS AND METHODS

Male Wistar rats (300–400 g) were used in this study. The rats were housed in groups of five and maintained in a temperature-controlled room (23 ± 1°C) with a 12/12 light–dark cycle; food (ground pellets) and water were available ad libitum. The body weight of each animal was recorded once a week throughout the experiment. The study was conducted in accordance with the ethical guidelines for investigations of experimental pain in conscious animals.[23] This research was approved by the institutional animal experimentation ethics committee.

The rats were randomly divided into four groups: group I (n = 9)—treated with saline intraperitoneal (IP) injections on days 1, 2, and 3; group II (n = 9)—treated with celecoxib (10 mg/kg) IP injections on days 1, 2, and 3; group III (n = 7)—treated with saline IP injections on days 1 to 14; and group IV (n = 7)—treated with celecoxib (10 mg/kg) IP injections on days 1 to 14.

Celecoxib (Pfizer, São Paulo, SP, Brazil) was freshly dissolved in saline and given by IP injection twice a day in a dose of 10 mg/kg and in a volume of 1 mL/kg. The first injection was made 2 hours before appliance placement, in order to test the preoperative use of the drug. The control groups received equivolumetric saline injections during the same period according to their experimental groups (celexoxib for 3 or 14 days).

The appliance design of this study follows that used by Leiker et al.[24] Animals were first placed under general anesthesia with xylazine (10 mg/kg) and ketamine (50 mg/kg). A closed coil nickel-titanium spring (Sentalloy, GAC, Ctr Islip, NY) calibrated to provide a force of 50 g was ligated to the maxillary first molar and connected to an orthodontic band cemented onto the incisors (Figure 1a). Previous studies have demonstrated that a 40–60 g level of force stimulated substantial molar tooth movement in rats.[25–27] A nickel–titanium spring was used to provide a relatively constant force level over the course of the experiment. After 14 days of tooth movement, the rats of all groups were decapitated and maxillae were excised.

The distance between the mesial surface of the first and the distal surface of the third molar was measured bilaterally with an electronic caliper for high accuracy (Digimatic-Mitutoyo, Telford, UK) under a dental operating microscope (DF Vasconcellos SA, São Paulo, SP, Brazil) at 16× magnification, improving the reliability of the method. Tooth movement was estimated by subtracting the mean of the repeated measured values from the untreated and treated sides (Figure 1b) as described by Hong et al.[28] The error of the method based on double measurements performed on 20 randomly selected animals was estimated by using Dahlberg's equation ($S = \sqrt{\Sigma\, d^2/2n}$, where n = number of paired measurements and d = deviations between the two measurements[29]). The error in measurement was 0.02 mm and was thus considered to be of no further importance.

The left hemimaxillae of five rats from each group were processed for tartrate-resistant acid phosphatase (TRAP) staining as described elsewhere.[30] For each sample three sagittal sections (5 μm thick) were taken at 50 μm intervals. The slides were counterstained with Harris's hematoxylin for 7 minutes. Cover slips were mounted with Entellan before examining the slides with a Leica Microsystems light microscope (Wetzlar, Germany).

In each section the osteoclasts were counted at the alveolar bone surface (compression side) adjacent to the entire mesial root. Cells were considered osteoclasts if they were multinucleated, TRAP positive, and located on or close to bone surfaces. The estimate of TRAP positive cells was determined by summing the

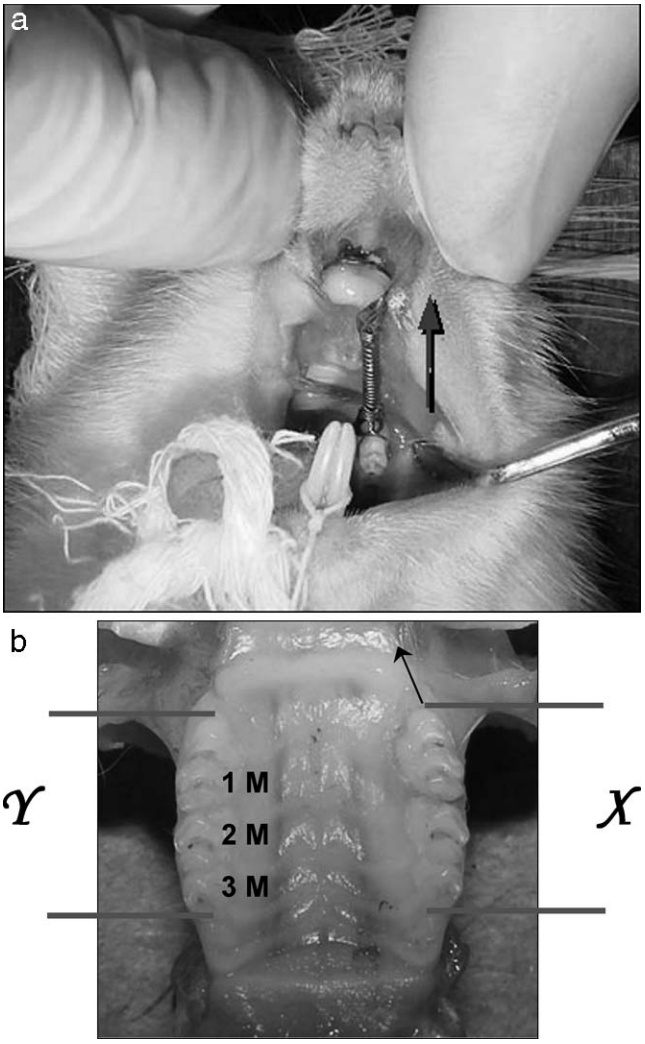

Figure 1. (a) Appliance used to move the molars mesially (arrows). (b) Indication of measurement procedure. The distance between the mesial side of the first molar (1M) and the distal side of the third molar (3M) was measured, and that of the treated side (X) subtracted from the one of the untreated side (Y).

value of the TRAP positive cells in the three sections per case. The mesial root was chosen because it is the largest of the five first molar roots, is in approximately the same plane as the applied force, and is most commonly evaluated in tooth movement studies.[24,31]

Table 2. Values (Median and Standard Deviation) for the Amount of Tooth Movement (mm) in Animals Treated with Saline or Celecoxib[a]

Drugs	Time of Treatment	
Saline	0.33 (0.12) Aa	0.28 (0.05) Ba
Celecoxib	0.23 (0.07) Ab	0.15 (0.06) Bb

[a] Distinct letters (capital letters for rows and small letters for columns) indicate statistical difference (two-way ANOVA and Tukey test, $P < .05$).

Cell counting was performed manually in a blinded manner, and a reproducibility error of less than 10% was established by recounting 20 randomly selected images. These counts were then compared with the original counts by using Dahlberg's equation. The error for TRAP variable was 0.64 and was thus considered acceptable.

Body weight was analyzed by repeated measures analysis of variance (ANOVA) and Tukey test. The amount of tooth movement and the number of osteoclasts were analyzed by two-way ANOVA and Tukey test. The SAS software (version 9.1, 2003; SAS Institute Inc, Cary, NC) was used and the significance level set at $P < .05$.

RESULTS

There was an overall gain in body weight over the two weeks of the experiment (Table 1; $P < .0001$). There was no statistical difference between groups ($P = .7632$), and the interaction between groups and weeks was not statistically significant ($P = .1520$).

The amount of tooth movement was significantly lower in the celecoxib-treated animals than in the control animals (Table 2; $P = .0009$). The difference between times of treatment was also significant ($P = .0430$). The interaction between drugs and time was not statistically significant ($P = .6025$).

The number of TRAP positive cells on the alveolar bone surface did not differ between drugs ($P = .1230$; Table 3), neither between times of treatment ($P = .4014$). Furthermore, the interaction between drugs and times was not statistically significant ($P = .3812$).

Table 1. Values (Median and Standard Deviation) for Body Weight (g) in the Four Groups Before the Experiment Began, After 1 Week, and at the End of the Experiment[a]

Group	n	Before	After 1 Week	After 2 Weeks
I – saline for 3 days	9	357.7 (30.6) Ca	363.3 (31.7) Ba	378.4 (33.7) Aa
II – celecoxib for 3 days	9	360.5 (27.4) Ca	371.8 (27.2) Ba	376.2 (25.5) Aa
III – saline for 14 days	7	359.5 (32.8) Ca	359.7 (32.6) Ba	366.2 (33.8) Aa
IV – celecoxib for 14 days	7	369.1 (20.8) Ca	380.5 (16.8) Ba	383.0 (30.0) Aa

[a] Distinct letters (capital letters for rows and small letters for columns) indicate statistical difference (repeated measures ANOVA and Tukey test, $P < .05$).

DISCUSSION

There have been reports on the effectiveness of NSAIDs in relieving the pain induced by orthodontic force activation.[13–15] However, some of these drugs can interfere with tooth movement.[14] As a result, the use of selective COX-2 inhibitors is increasing, replacing conventional NSAIDs in clinical practice,[6–8,22] although the specificity of coxibs can account for different effects of these drugs on tooth movement.[11]

In the present study, the amount of tooth movement and the associated bone resorption process were evaluated in rats submitted to short- and long-term celecoxib administration. Celecoxib was chosen in this study because this drug is the most common alternative to rofecoxib.[32] The short-term treatment was chosen in this study to mimic the preemptive or preoperative administration of analgesics to decrease postoperative pain, which has become the focus of recent research in orthodontics.[15,21,22] Some authors also recommended two postoperative doses, in addition to a preoperative dose, for a complete pain control during each orthodontic appointment.[15] Since the orthodontic pain will usually last for 2–3 days,[33] we used one preemptive dose followed by postoperative doses for 2 days. The long-term treatment was chosen to mimic a situation in patients undergoing celecoxib treatment during all days of tooth movement, which can occur in the treatment of chronic diseases. The dose of 10 mg/kg was chosen based on the literature experience,[34,35] and the protocol of administration (twice a day) was chosen considering the pharmacodynamics of celecoxib.[36] Our results showed that both the short- and long-term therapy with celecoxib significantly reduced the amount of tooth movement (Table 2).

Previous studies have shown that celecoxib did not interfere with tooth movement in rats.[11,18] Recently, de Carlos et al[11] showed that celecoxib and parecoxib, but not rofecoxib, are appropriate for discomfort and pain relief while avoiding interference during orthodontic tooth movement. In addition, Jerome et al[18] showed that celecoxib (Celebrex 50 mg/kg) given to rats in their drinking water did not affect tooth movement and appeared to offer some slight protection against root resorption. Our results did not confirm these findings.

The present study showed that both short- and long-term celecoxib administration were able to reduce significantly the amount of orthodontic tooth movement. The differences between the results of these studies could be due to the dosage, time interval of administration, and methodology of tooth movement analysis, which were not the same. The study of de Carlos et al[11] used local injections (in the maxillary gingiva, close to the first molar) on the day of appliance placement and after 3 and 5 days. Jerome et al[18] used celecoxib given to rats in their drinking water, which made it difficult to control drug ingestion. Since preoperative administration of analgesics for controlling orthodontic pain is increasing,[15,21,22] we wanted to mimic this situation, by administering celecoxib 2 hours before appliance placement, followed by 2 more days of medication, as suggested by clinical research.[15]

Table 3. Values (Median and Standard Deviation) for the Number of Tartrate-Resistant Acid Phosphatase (TRAP)-Positive Cells on Alveolar Bone Surface in Animals Treated with Saline or Celecoxib[a]

	Time of Treatment	
Drugs	3 Days	14 Days
Saline	28.8 (15.3) Aa	29.0 (13.0) Aa
Celecoxib	25.0 (10.8) Aa	15.8 (4.7) Aa

[a] Distinct letters (capital letters for rows and small letters for columns) indicate statistical difference (Two-way ANOVA, $P < .05$).

It is known that when an orthodontic force activates the microenvironment of periodontal tissue, several key proinflammatory cytokines are rapidly produced to trigger a cascade of cellular events involved in the tooth displacement.[37] Therefore, the use of preoperative analgesics, followed by 2 days of medication, can both reduce orthodontic pain[15,21,22] and the associated inflammatory and tooth movement processes, as observed in the animals treated with the short-term therapy (Table 2). Moreover, long-term celecoxib administration, which is commonly used to treat many different diseases,[16,17] can also reduce the rate of tooth movement, and this reduction was more evident than that observed in the short-term therapy (Table 2).

According to our knowledge, this study is the first to examine the effects of preoperative doses and the long-term use of coxibs on orthodontic tooth movement. The lack of statistical significance for the number of osteoclasts on the alveolar bone surface was unexpected. Although the celecoxib-treated animals showed a reduction in osteoclasts when compared with saline-treated animals (Table 3), these differences were not statistically significant. This does not exclude the possibility that celecoxib administration may affect the osteoclast activity induced by orthodontic appliance. Some studies demonstrate that the size of the osteoclasts and activity of the proton pump is also related to the ability of the individual osteoclast to resorb bone.[38,39] Thus, the trend toward a reduction in the number of osteoclasts observed in the present study indicates that our results need to be reevaluated and confirmed under other experimental sets, including the evaluation of osteoclastogenesis, cell fusion, acidification, and resorptive activity. Sari et al[40] showed that rofecoxib administration did not significantly affect PGE2 levels. However, our study used celecoxib, and the drug could affect other factors, such

as interleukin-1 and -6, which are both related to bone resorption and tooth movement.[37]

The significant difference in amount of tooth movement between animals treated with saline for 3 and 14 days (Table 2) was unexpected. One possible explanation could be the stress system response evoked by two injections/day during the 14 days of treatment. Kalia et al[41] showed that the administration of glucocorticoids can reduce bone turnover induced by orthodontic forces. Perhaps the endogenous glucocorticoids secreted by the rats submitted to repeated injections might be enough to interfere in the tooth movement.

CONCLUSIONS

- Orthodontists should be aware of patients under short- and long-term therapy with celecoxib, since this drug can slow down the rate of orthodontic tooth movement.
- Perhaps the use of other drugs or other administration protocols might be effective for discomfort and pain relief while avoiding interference during tooth movement.

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