ORIGINAL ARTICLE

GH Gameiro
DF Nouer
JS Pereira-Neto
MBB de Araújo Magnani
ED de Andrade
PD Novaes
MCF de Arruda Veiga

Histological analysis of orthodontic root resorption in rats treated with the cyclooxygenase-2 (COX-2) inhibitor celecoxib

Authors' affiliations:
Gustavo Hauber Gameiro, Darcy Flávio Nouer, João Sarmento Pereira-Neto, Maria Beatriz Borges de Araújo Magnani, Division of Orthodontics, Piracicaba Dental School, State University of Campinas – UNICAMP, Piracicaba, Brazil
Eduardo Dias de Andrade, Pharmacology, Piracicaba Dental School, State University of Campinas – UNICAMP, Piracicaba, Brazil
Pedro Duarte Novaes, Histology, Piracicaba Dental School, State University of Campinas – UNICAMP, Piracicaba, Brazil
Maria Cecília Ferraz de Arruda Veiga, Oral Physiology, Piracicaba Dental School, State University of Campinas – UNICAMP, Piracicaba, Brazil

Correspondence to:
Gustavo Hauber Gameiro
Division of Orthodontics
Piracicaba Dental School
University of Campinas – UNICAMP
Av. Limeira 901 C.P. 52, CEP 13414-900
Piracicaba, São Paulo, Brazil
E-mail: gustavo@gameiro.pro.br

Dates:
Accepted 16 January 2008

To cite this article:
Gameiro GH, Nouer DF, Pereira-Neto JS, de Araújo Magnani MBB, de Andrade ED, Novaes PD, de Arruda Veiga MCF:
Histological analysis of orthodontic root resorption in rats treated with the cyclooxygenase-2 (COX-2) inhibitor celecoxib
Orthod Craniofac Res 2008;**11**:156–161

Structured Abstract

Authors – Gameiro GH, Nouer DF, Pereira-Neto JS, de Araújo Magnani MBB, de Andrade ED, Novaes PD, de Arruda Veiga MCF

Introduction – It has been reported that anti-inflammatory drugs used for treatment of pain and discomfort related to orthodontic treatment could slow down tooth movement. However, the effect of these drugs on orthodontic root resorption is not well understood.

Objectives – The aim of this study was to investigate whether the COX-2 inhibitor celecoxib offers some protection against orthodontically induced root resorption.

Design – Male Wistar rats were divided into four groups: Groups I and II were treated with saline and celecoxib (10 mg/kg), respectively for 3 days. Groups III and IV were treated with saline and celecoxib for 14 days. The upper left first molars of all rats were moved mesially for 14 days with 50 *g* of force. An area including the disto-apical aspect of the mesial root of the first molar was processed for histological and histochemical techniques with tartarate-resistant acid phosphatase (TRAP).

Outcome Measure – The degree of root resorption was measured using an image analysis system with a grid-sheet superimposed in the root were resorption lacunae were counted. The number of TRAP-positive cells on the tooth root surface defined as odontoclasts were also evaluated.

Results – The results revealed that there were no significant differences in the degree of root resorption and in the number of odontoclasts on the root between the four groups studied.

Conclusion – The short and long-term celecoxib administration did not suppress the root resorption in case of experimental orthodontic force application.

Key words: celecoxib; cyclooxygenase 2 inhibitors; orthodontics; root resorption

Introduction

Root resorption is a partial loss of tooth root cementum and dentin and is one of the most frequent iatrogenic problems in orthodontic tooth movement (1). The cause of root resorption is considered to be multi-factorial and the causes are basically divided into mechanical (treatment-related) and biological (patient-related) factors or a combi-

nation of both (2). For the mechanical factors, a meta-analysis study showed that the total distance the dental apex has moved and the time it takes are correlated with the extent of an evident root resorption (2). The main biological factors for root resorption include the individual susceptibility on a genetic basis (3), some systemic diseases (4) and anomalies in root morphology (5). However, exact mechanisms of root resorption have not yet been fully understood.

The cells responsible for root resorption are called odontoclasts and these cells are considered to be types of cells similar to the osteoclasts (6). Odontoclasts are attached to the tooth root and are activated to resorb the matrix (7). Several approaches to prevent root resorption during orthodontic tooth movement were reported including the administration of L-thyroxine (8), bisphosphonates (9), doxyciline (10), echistatin, (11), and others (6, 12). However, the effects of these drugs on root resorption are still controversial. For example, some studies revealed that bisphosphonates which are potent inhibitors of bone resorption caused a significant dose-dependent inhibition of root resorption in rats after force application (9, 13, 14). On the other hand, others studies have reported increased root resorption with bisphosphonate treatment (15, 16). Controversy also exists as to the effects of corticosteroids on orthodontic root resorption. The acute administration of high (15 mg/kg) and therapeutic dosages (8 mg/kg) of glucocorticoids produced an increased orthodontic root resorption in rabbits (17) and rats (18), respectively. In contrast, Ong et al. (19) reported a reduced root resorption in rats treated with a lower dosage of corticosteroid (1 mg/kg).

Recently, Jerome et al. (20) showed that the selective cyclo-oxygenase (COX-2) inhibitor celecoxib (Celebrex 50 mg/kg) given to rats in their drinking water did not interfere with tooth movement and appeared to offer some slight protection against orthodontic root resorption. However, the method of administration might not be able to maintain significant plasma drug concentration and the clasts cells responsible for root resorption were not quantified. On this background, the present study was designed to investigate possible effects of the controlled celecoxib administration on the number of odontoclasts and on the degree of root resorption induced by orthodontic tooth movement.

Materials and methods

Animals

Male Wistar rats (3.5 month old, weighing 350 g on average) 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 and food (ground pellets) and water was 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 (21). This research was approved by the Institutional Ethics Committee in animal experimentation.

The rats were randomly divided into four groups: Group I (n = 9) – treated with saline i.p. injections on days 1, 2, and 3; Group II (n = 9) – treated with celecoxib (10 mg/kg) i.p. injections on days 1, 2, and 3. Group III (n = 7) – treated with saline i.p. injections on days 1 to 14; Group IV (n = 7) – treated with celecoxib (10 mg/kg) i.p. injections on days 1 to 14. The asymmetric distribution of the animals was because of the fact that two rats in group III died under general anesthesia and two rats in group IV had to be excluded because their appliances were damaged during the experiment.

Drug treatment

Celecoxib (Pfizer, São Paulo, Brazil) was freshly dissolved in saline and given i.p. twice a day in a dose of 10 mg/kg and in a volume of 1 ml/kg. The first injection was made 2 h before appliance placement to test the pre-emptive or pre-operative use of the drug which is the current trend in the orthodontic pain management (22, 23). The control groups received equivolumetric saline injections during the same period according to their experimental groups (Celexoxib for 3 or 14 days).

Appliance placement

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, NY, USA) calibrated to provide

a force of 50 *g* was ligated to the upper left first molar and connected to an orthodontic band cement onto the incisors. Previous studies have demonstrated that a 40–60 *g* level of force stimulated substantial molar tooth movement in rats (25, 26). A nickel–titanium spring was used to provide a relatively constant force level over the course of the tooth movement period (14 days).

Tartrate-resistant acid phosphatase histochemistry and evaluation of root resorption score

The left hemi-maxilla of five rats from each group was processed for tartrate-resistant acid phosphatase (TRAP) staining as described elsewhere (19). For each sample, three sagittal sections (5 μm thick) were taken at 50 μm intervals. The slides were counterstained with Harris's hematoxilin for 7 min. Cover slips were mounted with Entellan before examining the slides with a Leica Microsystems light microscope (Wetzlar, Germany).

Counting of odontoclasts was performed in a selected section localized disto-apically to the mesial root (Fig. 1). The overall size of each measurement area was 1000 μm × 800 μm. The magnification used to view the selected area was achieved with an objective of 20× magnification. Cells were considered to be odontoclasts if they were multinucleated, TRAP-positive, and located on or close to root surface (Fig. 2). The estimate of TRAP-positive cells was determined by summing the value of the TRAP-positive cells in the three sections per case. The mesial root was chosen because it is the largest of the first molar's five roots, in approximately the same plane as the applied force and is most commonly evaluated in tooth movement studies (24, 27).

The root resorption scores were determined according to the method described by Lu et al. (28) with little modifications. Under 10× magnification, a selected area localized disto-apically to the mesial root was examined using an image analysis system (Leica Qwin, Leica, Bensheim, Germany). A grid-sheet (10 × 10) was superimposed in the selected area (1000 μm × 800 μm) and the number of grids with or without resorption lacunae was counted (Fig. 3). Root resorption scores (percentage of resorption grids) were determined by dividing the number of grids with resorption lacunae by the total number of grids along the root surface (Fig. 3).

To determine the random intra-individual error for the odontoclasts counts and root resorption scores, 20 randomly chosen sections were counted again in a blinded manner and Dahlberg's equation was used. A reproducibility error of less than 10% was established and the errors for odontoclasts counts and root resporption scores were 0.47 and 3.1, respectively, which were considered to be acceptable.

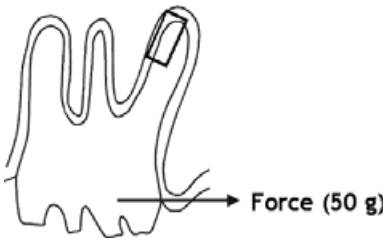

Fig. 1. The area under investigation (black rectangle) where tartrate-resistant acid phosphatase-positive cells were counted and root resorption scores were analyzed. The arrow indicates the direction of orthodontic force applied for 14 days.

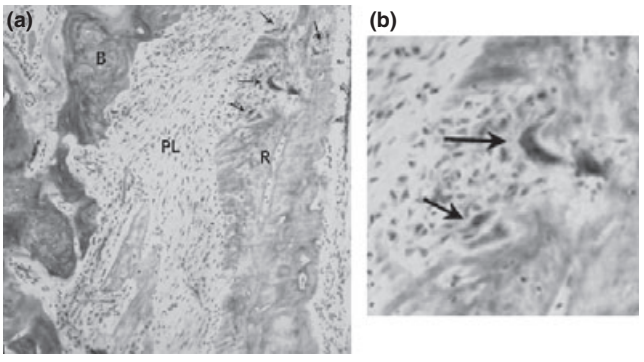

Fig. 2. Tartrate-resistant acid phosphatase stained histological sections taken from the disto-apical area of mesial root of the maxillary first molar (magnification 10 ×). (a) Details of odontoclasts cells are in (b). B, bone; PL, periodontal ligament; R, root.

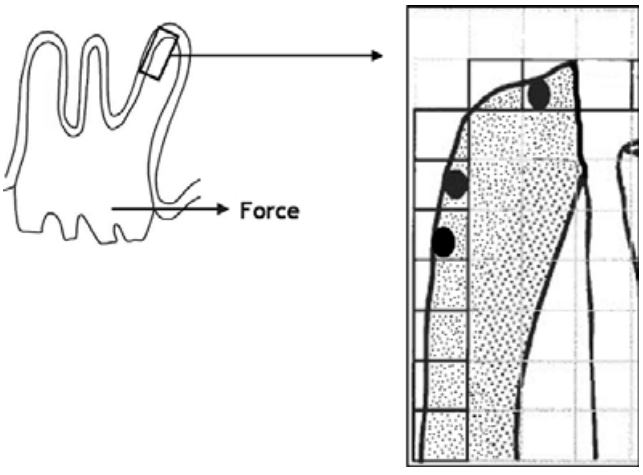

Fig. 3. Schematic illustration of the evaluation of root resorption score. Root resorption score = number of grids containing resorption lacunae (black circles) divided by the total number of grids along the root surface (black squares; 100 ×).

Statistical analysis

Body weight was analyzed by repeated measures ANOVA and Tukey's test. The odontoclasts number and root resorption scores were analyzed by two-way ANOVA and Tukey's test. The SAS software (version 9.1, 2003; SAS Institute Inc., Cary, NC, USA) was used and the significance level set at $p < 0.05$.

Results

Except for a temporary episode of weight loss in all animals for 1 to 2 days following appliance insertion, there was an overall gain in body weight over weeks ($p < 0.0001$). There was no statistical difference between groups ($p = 0.7632$) and the interaction between groups and weeks was also not statistically significant ($p = 0.1520$; data not shown).

The number of TRAP-positive cells on the root surface did not differ between drugs ($p = 0.7285$) neither between times of treatment ($p = 0.3721$). Also, the interaction between drugs and time points was not statistically significant ($p = 0.8348$; Table 1).

The values for root resorption score was similar between saline and celecoxib groups ($p = 0.7607$). There was no significant differences between times of treatment ($p = 0.8313$) and the interaction between drugs and times was also not statistically significant ($p = 0.1485$; Table 2).

Table 1. **Values (median and standard deviation) for the number of tartrate-resistant acid phosphatase-positive cells on the root surface in animals treated with saline or celecoxib**

	Time of treatment	
Drugs	3 days	14 days
Saline	11.8 (7.8)*,**	15.0 (3.0) *,**
Celecoxib	11.4 (5.3) *,**	13.4 (7.8) *,**

Means followed by the same symbols (* for rows and ** for columns) do not differ significantly (two-way ANOVA, $p < 0.05$).

Table 2. **Values (median and standard deviation) for the root resorption score in animals treated with saline or celecoxib**

	Time of treatment	
Drugs	3 days	14 days
Saline	40.9 (8.68) *,**	49.6 (9.15) *,**
Celecoxib	50.1 (13.6) *,**	43.6 (12.5) *,**

Means followed by the same symbols (* for rows and ** for columns) do not differ significantly (two-way ANOVA, $p < 0.05$).

Discussion

The existing literature supports the use of non-steroidal anti-inflammatory drugs (NSAIDs) for orthodontic pain control (29, 30) even though some of these drugs can impair bone resorption and hence the tooth movement (29). One approach to deal with this problem is the use of selective COX-2 inhibitors, which are replacing conventional NSAIDs in the clinical practice (23, 31, 32). It has previously been shown that some COX-2 inhibitors (Celecoxib and Parecoxib) do not interfere in the rate of orthodontic tooth movement (20, 33). However, the specificity of COX inhibitors can account for different effects of these drugs on tooth movement (33). One example is the Rofecoxib, a drug that can disturb the process of tooth movement (33, 34). In addition, this drug has been the object of debate and even withdrawn from the market because of reports of unwanted side effects. Celecoxib was the first COX-2 inhibitor introduced in the market and it still remains so, whereas rofecoxib and valdecoxib were withdrawn because of excess cardiovascular risk (35). To our knowledge, the possibility of using selective COX-2 inhibitors to prevent root resorption was reported only once (20). The present study was designed to evaluate the orthodontic root resorption in rats treated with short and long-term celecoxib administration.

The short-term treatment was chosen in this study to simulate the administration of pre-emptive or pre-operative analgesics to decrease post-operative pain, which has become the focus of recent research in orthodontics (22, 23). It is assumed that pre-emptive analgesia will block the afferent nerve impulses before they reach the central nervous system, abolishing the process of central sensitization (22). Some authors also recommended two post-operative doses, in addition to a pre-operative dose, for a complete pain control during each orthodontic appointment (30). As the orthodontic pain usually lasts for 2–3 days (32), we have used one pre-emptive dose followed by post-operative 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 (36). The dose of 10 mg/kg was chosen based on the literature experience (37, 38), and the protocol of administration (twice a day) was chosen considering the pharmacodynamics of celecoxib (39). Our results showed that not only the short-term therapy but also the long-term therapy with celecoxib did not affect the orthodontic root resorption, as confirmed by the absence of statistical difference between the number of odontoclasts on the root surface of the four studied groups (Table 1). Moreover, the short- and long-term celecoxib administration was not able to alter the root resorption scores, as observed when the medicated and control groups were compared (Table 2).

Jerome et al. used celecoxib (Celebrex 50 mg/kg) given to rats in their drinking water and found a significantly lower number of root lacunaes formed as consequence of orthodontic forces (20). However, no information about the control of drug ingestion was reported and direct comparison with our data is impossible. Moreover, the dosage and time interval of administration and methodology of root resorption analysis were not the same. We wanted to simulate the short and long-term use of celecoxib in the clinical practice. Although we have previously observed that the protocol of drug administration used in this study (with the same dosage) was able to reduce the rate of tooth movement in rats (40), it is well known that osteoclasts and odontoclasts differ in their susceptibility to modulation by pharmacologic agents (13–16, 18, 19). In contrast to alveolar bone, which is rich in cells and vessels, tooth cementum and dentin are largely matrix-dominant tissue with no vessels. Thus, dental tissues may not be affected by drugs and systemic factors compared with the alveolar bone. This observation is consistent with previously reported findings (41, 42).

Conclusions

The results of the present investigation indicated that the short and long-term administration of celecoxib did not reduce the odontoclasts and root resorption induced by orthodontic force application. These results need to be re-evaluated and confirmed under other experimental sets. Perhaps, the use of other COX-2 inhibitors or other drugs might be effective in protecting from root resorption induced by orthodontic treatment. Together with the evidence that celecoxib can slow down tooth movement, the results of the present study do not support the prescription of this drug to orthodontic patients.

Acknowledgements: Celecoxib was kindly provided by Pfizer (São Paulo, Brazil). The authors thank Gláucia Maria Bovi Ambrosano for statistical analyses. They thank Maria Aparecida Santiago Varella and Eliene Aparecida Orsini Narvaes Romani for their technical assistance. This work was supported by CAPES and FAPESP, Brazil.

References

1. Brezniak N, Wasserstein A. Orthodontically induced inflammatory root resorption. Part I: the basic science aspects. *Angle Orthod* 2002;72:175–9.
2. Segal GR, Schiffman PH, Tuncay OC. Meta analysis of the treatment-related factors of external apical root resorption. *Orthod Craniofac Res* 2004;7:71–8.
3. Al-Qawasmi RA, Hartsfield JK Jr, Everett ET, Flury L, Liu L, Foroud TM et al. Genetic predisposition to external apical root resorption in orthodontic patients: linkage of chromosome 18 marker. *J Dent Res* 2003;82:356–60.
4. Nishioka M, Ioi H, Nakata S, Nakasima A, Counts A. Root resorption and immune system factors in the Japanese. *Angle Orthod* 2006;76:103–8.
5. Sameshima GT, Sinclair PM. Predicting and preventing root resorption. Part I. Diagnostic factors. *Am J Orthod Dentofacial Orthop* 2001;119:505–10.
6. Jager A, Zhang D, Kawarizadeh A, Tolba R, Braumann B, Lossdorfer S et al. Soluble cytokine receptor treatment in experimental orthodontic tooth movement in the rat. *Eur J Orthod* 2005;27:1–11.
7. Kuroda S, Balam TA, Sakai Y, Tamamura N, Takano-Yamamoto T. Expression of osteopontin mRNA in odontoclasts revealed by in situ hybridization during experimental tooth movement in mice. *J Bone Miner Metab* 2005;23:110–3.
8. Loberg EL, Engstrom C. Thyroid administration to reduce root resorption. *Angle Orthod* 1994;64:395–9; discussion 399–400.
9. Igarashi K, Adachi H, Mitani H, Shinoda H. Inhibitory effect of the topical administration of a bisphosphonate (risedronate) on root resorption incident to orthodontic tooth movement in rats. *J Dent Res* 1996;75:1644–9.
10. Mavragani M, Brudvik P, Selvig KA. Orthodontically induced root and alveolar bone resorption: inhibitory effect of systemic doxycycline administration in rats. *Eur J Orthod* 2005;27:215–25.
11. Talic NF, Evans C, Zaki AM. Inhibition of orthodontically induced root resorption with echistatin, an RGD-containing peptide. *Am J Orthod Dentofacial Orthop* 2006;129:252–60.
12. Chung CJ, Soma K, Rittling SR, Denhardt DT, Hayata T, Nakashima K et al. OPN deficiency suppresses appearance of odontoclastic cells and resorption of the tooth root induced by experimental force application. *J Cell Physiol* 2008;214:614–20.

13. Adachi H, Igarashi K, Mitani H, Shinoda H. Effects of topical administration of a bisphosphonate (risedronate) on orthodontic tooth movements in rats. *J Dent Res* 1994;73:1478–86.
14. Igarashi K, Mitani H, Adachi H, Shinoda H. Anchorage and retentive effects of a bisphosphonate (AHBuBP) on tooth movements in rats. *Am J Orthod Dentofac Orthop* 1994;106:279–89.
15. Alatli I, Hellsing E, Hammarstrom L. Orthodontically induced root resorption in rat molars after 1-hydroxyethylidene-1,1-bisphosphonate injection. *Acta Odontol Scand* 1996;54:102–8.
16. Alatli I, Hammarstrom L. Root surface defects in rat molar induced by 1-hydroxyethylidene-1,1-bisphosphonate. *Acta Odontol Scand* 1996;54:59–65.
17. Ashcraft MB, Southard KA, Tolley EA. The effect of corticosteroid-induced osteoporosis on orthodontic tooth movement. *Am J Orthod Dentofacial Orthop* 1992;102:310–9.
18. Verna C, Hartig LE, Kalia S, Melsen B. Influence of steroid drugs on orthodontically induced root resorption. *Orthod Craniofac Res* 2006;9:57–62.
19. Ong CK, Walsh LJ, Harbrow D, Taverne AA, Symons AL. Orthodontic tooth movement in the prednisolone-treated rat. *Angle Orthod* 2000;70:118–25.
20. Jerome J, Brunson T, Takeoka G, Foster C, Moon HB, Grageda E et al. Celebrex offers a small protection from root resorption associated with orthodontic movement. *J Calif Dent Assoc* 2005;33:951–9.
21. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 1983;16:109–10.
22. Bernhardt MK, Southard KA, Batterson KD, Logan HL, Baker KA, Jakobsen JR. The effect of preemptive and/or postoperative ibuprofen therapy for orthodontic pain. *Am J Orthod Dentofacial Orthop* 2001;120:20–7.
23. Young AN, Taylor RW, Taylor SE, Linnebur SA, Buschang PH. Evaluation of preemptive valdecoxib therapy on initial archwire placement discomfort in adults. *Angle Orthod* 2006;76:251–9.
24. Leiker BJ, Nanda RS, Currier GF, Howes RI, Sinha PK. The effects of exogenous prostaglandins on orthodontic tooth movement in rats. *Am J Orthod Dentofacial Orthop* 1995;108:380–8.
25. King GJ, Keeling SD, McCoy EA, Ward TH. Measuring dental drift and orthodontic tooth movement in response to various initial forces in adult rats. *Am J Orthod Dentofacial Orthop* 1991;99:456–65.
26. Rody WJJ, King GJ, Gu G. Osteoclast recruitment to sites of compression in orthodontic tooth movement. *Am J Orthod Dentofacial Orthop* 2001;120:477–89.
27. Kalia S, Melsen B, Verna C. Tissue reaction to orthodontic tooth movement in acute and chronic corticosteroid treatment. *Orthod Craniofac Res* 2004;7:26–34.
28. Lu LH, Lee K, Imoto S, Kyomen S, Tanne K. Histological and histochemical quantification of root resorption incident to the application of intrusive force to rat molars. *Eur J Orthod* 1999;21:57–63.
29. Arias OR, Marquez-Orozco MC. Aspirin, acetaminophen, and ibuprofen: their effects on orthodontic tooth movement. *Am J Orthod Dentofacial Orthop* 2006;130:364–70.
30. Polat O, Karaman AI, Durmus E. Effects of preoperative ibuprofen and naproxen sodium on orthodontic pain. *Angle Orthod* 2005;75:791–6.
31. Gameiro GH, Pereira-Neto JS, Magnani MB, Nouer DF. The influence of drugs and systemic factors on orthodontic tooth movement. *J Clin Orthod* 2007;41:73–8.
32. Krishnan V. Orthodontic pain: from causes to management – a review. *Eur J Orthod* 2007;29:170–9.
33. de Carlos F, Cobo J, Perillan C, Garcia MA, Arguelles J, Vijande M et al. Orthodontic tooth movement after different coxib therapies. *Eur J Orthod* 2007;29:596–9.
34. de Carlos F, Cobo J, Diaz-Esnal B, Arguelles J, Vijande M, Costales M. Orthodontic tooth movement after inhibition of cyclooxygenase-2. *Am J Orthod Dentofacial Orthop* 2006;129:402–6.
35. Antoniou K, Malamas M, Drosos AA. Clinical pharmacology of celecoxib, a COX-2 selective inhibitor. *Expert Opin Pharmacother* 2007;8:1719–32.
36. Rattray B, Nugent DJ, Young G. Celecoxib in the treatment of haemophilic synovitis, target joints, and pain in adults and children with haemophilia. *Haemophilia* 2006;12:514–7.
37. Fabricio AS, Veiga FH, Cristofoletti R, Navarra P, Souza GE. The effects of selective and nonselective cyclooxygenase inhibitors on endothelin-1-induced fever in rats. *Am J Physiol Regul Integr Comp Physiol* 2005;288:R671–7.
38. Mizuno M, Sotoyama H, Narita E, Kawamura H, Namba H, Zheng Y et al. A cyclooxygenase-2 inhibitor ameliorates behavioral impairments induced by striatal administration of epidermal growth factor. *J Neurosci* 2007;27:10116–27.
39. Davies NM, McLachlan AJ, Day RO, Williams KM. Clinical pharmacokinetics and pharmacodynamics of celecoxib: a selective cyclo-oxygenase-2 inhibitor. *Clin Pharmacokinet* 2000;38:225–42.
40. Gameiro GH, Nouer DF, Neto JSP, Siqueira VC, Andrade ED, Novaes PD et al. Effects of short and long-term celecoxib on orthodontic tooth movement. *Angle Orthod* 2008;78:861–6.
41. Goldie RS, King GJ. Root resorption and tooth movement in orthodontically treated, calcium-deficient, and lactating rats. *Am J Orthod* 1984;85:424–30.
42. Midgett RJ, Shaye R, Fruge JF Jr. The effect of altered bone metabolism on orthodontic tooth movement. *Am J Orthod* 1981;80:256–62.