

# The Effect of Orthodontic Forces on Calcitonin Gene-related Peptide Expression in Human Dental Pulp

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## Abstract

**Introduction:** The purpose of this study was to quantify the effect of moderate and severe orthodontic forces on calcitonin gene-related peptide (CGRP) expression in healthy human dental pulp. **Methods:** Thirty human dental pulp samples were obtained from healthy premolars in which extraction was indicated for orthodontic reasons. Before extraction, teeth were divided into 3 groups of 10 premolars each: (1) the control group: healthy premolars without application of orthodontic forces; (2) the moderate force group: a 56-g force was applied to the premolars for 24 hours; and (3) the severe force group: a 224-g force was applied to the premolars for 24 hours. All dental pulp samples were processed, and CGRP was measured by radioimmunoassay. **Results:** Greater CGRP expression was found in the severe force group followed by the moderate force group. The lower CGRP values were for the control group. The Kruskal-Wallis test showed statistically significant differences between groups ( $P < .0001$ ). Least significant difference (LSD) post hoc tests showed statistically significant differences in CGRP expression between the control group and the severe force group ( $P < .0001$ ) but not with the moderate force group ( $P = .06$ ). Differences between the moderate and severe force groups were statistically significant ( $P < .0001$ ). **Conclusions:** CGRP expression in human dental pulp increases when teeth are submitted to severe orthodontic forces. (*J Endod* 2011;37:934–937)

## Key Words

Calcitonin gene-related peptide, human dental pulp, orthodontic forces

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Orthodontic movement has been associated with some alterations to the pulpodentin complex such as disruption of the odontoblastic layer, alteration of pulp microcirculation, hypoxia, and pulp calcifications (1–3). It has been shown that depending on the duration, type, and magnitude of the force as well as physiological tissue tolerance, these alterations could affect the pulp tissue in a reversible or irreversible manner (4). The extent of dental pulp injury is also correlated to the degree of the inflammatory response generated, which is mediated by neuropeptides (5).

Calcitonin gene-related peptide (CGRP) is a neuropeptide released from pulp C-type nerve fibers after being injured. CGRP is capable of triggering vasodilation; plasma extravasation; immune system activation; chemotaxis; and recruitment and/or regulation of inflammatory cells such as macrophages, mast cells, and lymphocytes (6). The changes in pulp microcirculation elevate tissue pressure, which may account for degenerative reactions such as pulp calcification or pulp necrosis (4).

Previous studies have associated extrusive orthodontic forces with some deleterious effects in dental pulp and periodontal ligament, ranging from vascular stasis to pulp necrosis (7, 8). Different range forces have been recommended in adults to prevent deleterious effects on the dental pulp, showing that the optimal range lies between 50 and 100 cN (25–50 g). However, the 100- to 150-cN (50–75 g) range has also been suggested. Orthodontic forces range between 150 and 200 cN (75–100 g), and higher values are capable of generating pulp and periodontal ligament damage (9).

The effect of intrusive forces in a range of 35 to 250 g during 4 to 35 days has also been studied, showing a histological disruption in the odontoblastic layer, which was more evident in mature than immature teeth, suggesting that an alteration in pulp microcirculation could be responsible for this alteration and that tissue damage is directly related to the magnitude and direction of the orthodontic force (8, 9). Pulp neurogenic inflammation may lead to a fibrotic degeneration and/or pulp calcification affecting pulp vitality. If the application of the orthodontic force extends beyond tissue tolerance, pulp may get damaged to an irreversible state, leading to necrosis (10).

Up to date, no studies using human dental pulp samples have investigated the effect of orthodontic forces on CGRP expression. The quantification of this neuropeptide in human dental pulp will provide scientific knowledge that could help explain the biological mechanisms involved during the pulpal response to orthodontic movement. Therefore, the purpose of this article was to determine the expression of CGRP in human dental pulp of teeth subjected to moderate and severe orthodontic forces.

## Materials and Methods

An experimental study was performed according to Colombian Ministry of Health recommendations regarding ethical issues in research involving human tissue. Written informed consent was obtained from each of the patients participating in the study (18–37 years old, healthy, not medicated, and nonsmoking human donors, with premolars extraction indicated for orthodontic reasons). All teeth used were caries and restoration free with complete root development determined radiographically (and confirmed visually after extraction), without signs of periodontal disease or traumatic occlusion and without previous orthodontic forces.

## Experimental Procedure

Dental pulp samples were obtained from 30 premolars that were randomly divided into 3 groups of 10 premolars each as follows: (1) the normal control group (without orthodontic forces, (2) the moderate force group, and (3) the severe force group. Teeth in moderate and severe orthodontic force groups were submitted to tipping and extrusion orthodontic movements.

Before orthodontic force application, the occlusal surface of the first mandibular molar was raised with a block of resin (Filtek Z350; 3M Espe, Seefeld, Germany) until the premolars were out of occlusion. A convertible standard buccal tube (Orthoorganizer, Carlsbad, CA) was bonded over the buccal face of the first molar with resin (Light Bond; Reliance Orthodontic Products Inc, Itasca, IL). A McLaughlin, Bennett, and Trevisi (MBT) slot size 0.022 bracket (Ref. 702-393 MC, Orthoorganizer) was bonded over the buccal face of the premolars (Fig. 1). One  $0.0017 \times 0.025$  in titanium molybdenum alloy (TMA) wire cantilever was inserted into each first molar tube and the wire was bent buccally to form a helix.

The cantilever was clinched to the distal end of the tube and exerted a tipping and extrusive force on the premolar. For the teeth in the moderate force group, the activation angle was  $45^\circ$  with a force of 56 g. For the severe force group, the activation angle was  $90^\circ$  with a force of 224 g. For both groups, forces were measured with an orthodontic dynamometer (Fig. 2). Once the force was measured, the free end of the sectional arch was hooked to the bracket with a metallic ligature (Fig. 3). Twenty four hours after, the ligature, the sectional arch, the tube, and the resin block were removed in order to perform the extraction procedure.

All teeth were anesthetized with 1.8 mL 4% prilocaine without vasoconstrictor by infiltrative injection for upper premolars and inferior alveolar nerve block injection for lower premolars. Adequate pulpal anesthesia was ascertained with a negative response to an electronic pulp vitality test.

## Sample Collection

Teeth in the control and orthodontic forces group were extracted 10 minutes after anesthetic application with conventional methods and without excessive injury to the periodontal ligament. Immediately after extraction, teeth were sectioned using a Zekrya bur (Dentsply, Tulsa, OK) in a high-speed handpiece irrigated with saline solution. Pulp tissue was obtained using a sterile endodontic excavator, placed on an Eppendorf tube, snap frozen in liquid nitrogen, and kept at  $-70^\circ\text{C}$  until use.

## Radioimmunoassay

Dental pulp samples were defrosted without thermal shock, dried on a filter, and individually weighed on an analytical balance. Neuropeptide was extracted by adding  $150\ \mu\text{L}$  of 0.5 mol/L acetic acid and double boiling in a thermostat bath for 30 minutes in accordance with previously reported protocols (11–16).

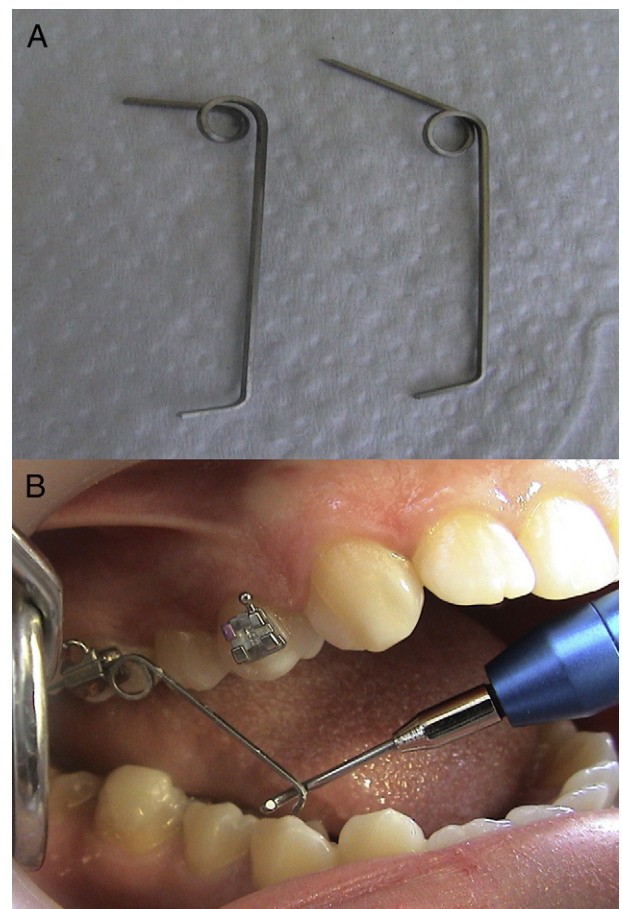
CGRP expression was determined by competition binding assays using a human CGRP-radioimmunoassay (RIA) kit from Phoenix Peptide Pharmaceutical (Ref. RK-015-02, Belmont, CA). Fifty microliters of each sample solution was incubated in polypropylene tubes at room temperature for 20 hours with  $100\ \mu\text{L}$  of primary antibody and  $100\ \mu\text{L}$  of different CGRP concentrations (10 pg/mL–1,280 pg/mL). Then,  $50\ \mu\text{L}$  of  $^{125}\text{I}$ -CGRP was added and left to incubate for another 24 hours. Bound fractions were precipitated by the addition of  $100\ \mu\text{L}$  of a secondary antibody (goat antirabbit immunoglobulin G serum),  $100\ \mu\text{L}$  of normal rabbit serum, and  $500\ \mu\text{L}$  of RIA buffer containing 1% polyethylene glycol 4000. After 2 hours of incubation at room temperature, tubes were spun at 3,000 rpm for 45 minutes at  $4^\circ\text{C}$ . The supernatants were decanted, and pellet radioactivity was read on a Gamma Counter (Gamma Assay LS 5500; Beckman, Fullerton, CA). Standard



**Figure 1.** Resin placed over the occlusal surface of the first mandibular molar to leave the premolars out of occlusion. A convertible standard buccal tube was bonded over the buccal face of the first molar and an MBT slot size 0.022 bracket was bonded over the buccal face of the premolars.

curves of authentic peptide were made in buffers identical to the tissue extracts on semilog graph paper.

Finally, analysis of the binding data assessed the amount of CGRP present in every sample, using the percentage of maximum binding (B/B0%) calculated for each unknown sample and reading across



**Figure 2.** (A) The TMA wire cantilever bent at  $90^\circ$  and  $45^\circ$ . (B) The cantilever was clinched to the distal end of the tube, and the orthodontic force was measured with an orthodontic dynamometer to 56 and 224 g.





**Figure 3.** The TMA wire cantilever in place to exert a tipping and extrusive force on the premolar.

the graph to the point of intersection with the calibration curve where the corresponding x-axis coordinate is equivalent to the concentration of peptide in the assayed sample.

## Statistical Analysis

Values are presented as CGRP concentration in picomoles per milligram of dental pulp tissue. Mean, standard deviation, medians, and maximum/minimum values are presented for each group. The Kruskal-Wallis test was performed to establish statistically significant differences between groups ( $P < .05$ ). Least significant difference (LSD) post hoc comparisons were also performed.

## Results

CGRP was found to be present in all dental pulp samples (Table 1). Highest CGRP levels were observed in the severe force group, with a mean value of  $0.1380 \pm 0.0278$  (median = 0.1386) pmol CGRP per mg of dental pulp followed by the moderate force group with a mean value of  $0.0609 \pm 0.0236$  (median = 0.0602) pmol CGRP per mg of dental pulp. Lowest CGRP levels were observed in the intact teeth control group samples with a mean value of  $0.0447 \pm 0.0109$  (median = 0.0434) pmol CGRP per mg of dental pulp.

The Kruskal-Wallis test showed statistically significant differences between groups ( $P < .0001$ ). LSD post hoc tests showed significant statistical differences between the intact-teeth control group and the severe force group ( $P < .0001$ ) but not with the moderate force group ( $P = .06$ ). Differences between the moderate and severe force groups were also statistically significant ( $P < .0001$ ).

## Discussion

Orthodontic movements have been considered to cause some inflammatory alterations in the dental pulp that are proportionally correlated with the magnitude, direction, and duration of the force

applied (9). Neuropeptides, including CGRP, released from C-type sensitive fibers upon stimulation by mechanical stimuli are capable of regulating the inflammatory reaction by controlling the vascular tone and blood flow, promoting a rapid and large arrival of immunocompetent cells and inflammatory mediators (6). During this process, pulp microcirculation undergoes dynamic changes that compromise its ability to remove metabolic waste products and to maintain a harmonic interstitial pressure. In consequence, pulp tissue edema and necrosis could take place (5).

CGRP values were obtained from healthy premolars in which extraction was indicated for orthodontic reasons. Before extraction, premolars in experimental groups were submitted to 24 hours of moderate or severe orthodontic force application. Both experimental groups used a cantilever made of  $0.017 \times 0.025$  TMA wire, which is ideal for the experiment because of its rectangular shape and its long transverse diameter that allows proper buccolingual control with an adequate elasticity module (17–20). The activation angle controls the direction of the force exerted by a cantilever. In the present research, it was determined that activation angles of  $45^\circ$  and  $90^\circ$  generate 56- and 224-g forces, respectively, as measured with a dynamometer. All orthodontic appliances were carefully designed, placed, and removed by one orthodontist.

The local anesthetic used in all groups of this study was 4% prilocaine without vasoconstrictor to prevent neuropeptide expression becoming attenuated by vasoconstrictors as previously shown (21, 22). The extraction procedure was also standardized to affect all teeth equally; it was performed in less than 5 minutes and without excessive injury to periodontal ligament.

Results from the present study showed that teeth that have been submitted to severe orthodontic forces experienced a significant increase in CGRP expression when compared with normal neuropeptide values. This finding supports the hypothesis that sensory nerve fibers respond to excessive orthodontic forces. CGRP released from nerve fibers can provoke a significant alteration in tissue homeostasis and pain sensitivity by triggering the release of inflammatory mediators (6, 23). If the orthodontic force is not eliminated, nerve fibers could become sensitized and therefore experience spontaneous depolarization, enhancing pain response (24).

CGRP increase in dental pulp suggests that orthodontic forces are capable of triggering a cellular response similar to the observed during caries progression, cavity preparation, and occlusal trauma (23, 25, 26). It has been reported that CGRP amplifies the inflammatory effects of substance P by increasing the release of inflammatory mediators and, thus, perpetuating this process leading to dental pulp degeneration such as calcification and/or necrosis (5, 6, 27, 28).

Evidence suggests that orthodontic forces affect the microcirculation of the pulp. Once the tissue is damaged, pulpal blood flow and collateral circulation become compromised, leading to hypoxia (29, 30). CGRP also increases the expression of bone morphogenetic protein-2 transcripts in human pulp cells, stimulating odontoblasts to increase dentin deposition as a defense mechanism, which in conjunction with hypoxia induces pulp tissue to undergo

**TABLE 1.** CGRP Expression in Human Dental Pulp from Healthy Human Premolars after Moderate and Severe Orthodontic Force Application

|                            | n  | Mean*  | Standard deviation | Median | Minimum | Maximum |
|----------------------------|----|--------|--------------------|--------|---------|---------|
| Control group <sup>†</sup> | 10 | 0.0447 | 0.0109             | 0.0434 | 0.0311  | 0.0664  |
| Moderate force             | 10 | 0.0609 | 0.0236             | 0.0602 | 0.0219  | 0.0937  |
| Severe force               | 10 | 0.1380 | 0.0278             | 0.1386 | 0.1000  | 0.1786  |

\*Values are presented as CGRP concentration in picomoles per milligram of dental pulp. The Kruskal-Wallis test showed statistically significant differences between groups ( $P < .0001$ ).

<sup>†</sup>The LSD post hoc test showed significant differences with the severe force group ( $P < .0001$ ) but not with the moderate force group ( $P = .06$ ). There was also a statistically significant difference between the moderate and severe force groups ( $P < .0001$ ).

a degenerative calcific reaction that could partially or completely obliterate pulp space (1, 31, 32).

There was also a slight nonsignificant increase in CGRP expression in the teeth submitted to moderate orthodontic forces that can be correlated with a lesser damage to pulp tissue. The minor alteration of pulpal blood flow and collateral circulation caused by vascular congestion initiates a compensatory mechanism that releases angiogenic growth factors to the extracellular space. These changes intend to avoid irreversible damage caused by a vascular breakdown (33, 34).

It is important to be aware that the present study only measured CGRP expression 24 hours after orthodontic force application and therefore reflects a model of acute inflammation. Because of the *in vivo* nature of the study and taking in consideration ethical issues, it would be difficult to develop a similar chronic inflammation model in which it would be necessary to leave the patients with orthodontic forces for a longer period of time (weeks or months), which may cause occlusal trauma, pain, and discomfort to the patients (35).

However, it can be hypothesized that in the long-term a severe orthodontic force may cause a magnification of the pulp inflammatory process that could lead to irreversible pulpitis and necrosis. Therefore, it is important to point out the clinical relevance of using moderate and intermittent orthodontic forces, which are capable of generating an adequate tooth movement, limiting the damage, and allowing pulp to recover from the injury (23). Therefore, it is advisable to use controlled movements and long resting periods in order to achieve the esthetic and functional objectives of orthodontic treatment, without triggering severe inflammatory reaction capable of inducing irreversible damage to the dental pulp and periapical tissues.

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