The Effect of Three Different Rotary Instrumentation Systems on Substance P and Calcitonin Gene-related Peptide Expression in Human Periodontal Ligament

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Abstract

Introduction: The purpose of this study was to quantify the effect of three different rotary root canal preparation systems on substance P and calcitonin gene-related peptide expression in healthy human periodontal ligament. **Methods:** Fifty periodontal ligament samples were obtained from healthy premolars in which extraction was indicated for orthodontic reasons. Before extraction, 40 of these premolars were equally divided into four groups, and root canals were prepared using four different systems: the ProTaper Universal rotary system, the RaCe rotary system, the Mtwo rotary system, and the hand instrumentation technique. The remaining 10 healthy premolars that were extracted without treatment served as a negative control group. All periodontal ligament samples were processed, and SP and CGRP were measured by radioimmunoassay. **Results:** Greater SP and CGRP expression were found in the ProTaper Universal group followed by the hand instrumentation group, the RaCe, and the Mtwo groups. The lower SP and CGRP values were for the negative control group. The Kruskal-Wallis test showed statistically significant differences between groups ($p < 0.0001$). Post hoc Least Significant Difference (LSD) tests showed statistically significant differences in SP and CGRP expression between the negative control group and all the other groups except the Mtwo group. Hand instrumentation also showed statistically significant differences with all the other groups, except the ProTaper Universal group. Differences between the three rotary systems were also statistically significant. **Conclusion:** SP and CGRP expression in periodontal ligament increases when teeth are prepared with ProTaper Universal and RaCe rotary instrumentation systems as well as with hand instrumentation. Mtwo maintains SP and CGRP levels. *(J Endod 2010;36:1938–1942)*

Key Words

Calcitonin gene-related peptide, human periodontal ligament, neurogenic inflammation, rotary instrumentation, substance P

A frequent problem in endodontics is the development of post-treatment symptomatic apical periodontitis, which may vary from a low-intensity sensitivity when biting over the tooth to a severe pain to even the slightest touch (1). Apical periodontitis is defined as a circumscribed inflammation of the periodontal ligament in the apical region and it has been reported to be provoked by extrusion of different irritants from the root canal system (such as dentin debris, necrotic tissue, microorganisms, irrigants and/or filling materials) towards the periapex during canal preparation, generating an antigen-antibody reaction with the correspondent inflammatory reaction, even when working length is well established (2, 3).

Similar to dental pulp, periodontal ligament inflammation has a neurogenic source, which is induced by the release of neuropeptides from periapical tissue C-type nerve fibers, after being injured during root canal therapy (4). Substance P (SP) and calcitonin gene-related peptide (CGRP) are capable of triggering vasodilation, plasma extravasation, immune system activation, chemotaxis, recruitment, and/or regulation of inflammatory cells such as macrophages, mast cells, and lymphocytes (5). Finally, the release of inflammatory mediators in the periodontal ligament generates vascular stasis in the affected area (6, 7). Recent evidence has suggested that human fibroblasts are able to produce SP and that neuropeptides could also regulate the expression of angiogenic growth factors in fibroblasts, suggesting that these cells also play a role in neurogenic inflammation (8, 9). These biological effects could explain the clinical events of pain and inflammation observed during symptomatic apical periodontitis after root canal therapy (10).

It has been reported that the severity of periodontal ligament inflammation is directly proportional to the degree of the tissue damage (ie, the quantity of apically extruded debris [1, 11] and the mechanical stress exerted on the tooth [12]). It also has been shown that all root canal preparation techniques cause some degree of debris extrusion (11, 13–16). However, the amount of apically extruded irritants may vary according to the technique and the characteristics of the instrument used (3).

According to manufacturers, nickel-titanium rotary instruments, such as ProTaper Universal (Dentsply Maillefer, Ballaigues, Switzerland), Mtwo (VDW, Munich, Germany) and RaCe (FKG, La Chaux-de-Fonds, Switzerland), have been designed with different physical characteristics (ie, profile section, core diameter, rake angle,

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variable helicoidal angle, variable distance between flutes, or pitch) in order to reduce the quantity of debris extruded into the periodontal ligament. It has been stated that the profile section of an instrument establish the size of its core and together with the distance between flutes are responsible of providing enough space to allow detritus to be removed coronally, therefore reducing apical extrusion (17). Moreover, instruments with a positive rake angle have more cutting effectiveness and suffer less torsional stress when working because they are not easily blocked, avoiding the instrument to pump debris into the periapical tissues and reducing the mechanical stress over the tooth (18). Taking into consideration that root canal preparation may trigger a neurogenic inflammation response in the periodontal ligament and that SP and CGRP play an important role during this inflammatory process, the purpose of this study was to quantify the effect of different root canal rotary instrumentation systems on SP and CGRP expression in healthy human periodontal ligament. This knowledge could be useful for assessing neuropeptide behavior when routine endodontic procedures are performed, and consequently, contribute to clinician's decision making to minimize tissue injury.

Materials and Methods

A descriptive comparative 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 patient participating in the study (18-30 years old, healthy, not medicated, and nonsmoking human donors). Fifty periodontal ligament samples were obtained from 50 lower premolars in which extraction was indicated for orthodontic reasons. All teeth used were caries and restoration free with complete root development determined both visually and radiographically, without signs of periodontal disease or traumatic occlusion and without orthodontic forces. Teeth had only one straight canal (canal curvatures over 20° were not included).

Teeth were equally divided and randomly assigned into the following five groups: (1) ProTaper Universal, (2) RaCe, (3) Mtwo, (4) hand instrumentation, and (5) intact-teeth control group. All teeth were anesthetized by an inferior alveolar nerve block injection of 1.8 mL 4% prilocaine without vasoconstrictor. Adequate pulpal anesthesia was ascertained with a negative response to an electronic pulp vitality test.

Experimental Procedure and Sample Collection

For the intact-teeth control group, extraction was performed by conventional methods without excessive injury to the periodontal ligament 10 minutes after anesthetic application. For the rest of the groups, teeth were isolated with a rubber dam, cavity accesses were performed using a Zekrya bur (Dentsply, Tulsa, OK) in a high-speed handpiece, the working length was established with the aid of an apex locator (Root ZX II, J Morita, Japan) set to 0.5 mm and radiographically confirmed, and finally root canals were prepared with the corresponding preparation technique before extraction as follows: (1) the ProTaper Universal group: root canals were prepared using the Protaper Universal (Denstply Maillefer, Ballaigues, Switzerland) technique strictly following manufacturer's sequence and recommendations (ie, SX, S1, and S2 with brushing motion and F1, F2, F3, and F4 until reaching the working length without apical pressure), (2) the RaCe group: root canals were prepared using the RaCe (FKG, La Chaux-de-Fonds, Switzerland) technique strictly following manufacturer's sequence and recommendations (ie, PreRaCe 40/.10 and 35/.08 for preflaring and RaCe 20/.02, 25/.02, 30/.04, 35/.04 and 40/.04 reaching the working length with a brushing motion and without apical pressure), (3) the Mtwo group: root canals were prepared using the Mtwo (VDW, Munich, Germany) technique strictly following manufacturer's sequence and recommendations (ie, 10/.04, 15/.05, 20/.06, 25/.06, 30/.05, 35/.04, and 40/.04, all of them reaching working length without apical pressure), and (4) the hand instrumentation group: root canals were prepared with hand instrumentation using Flexofiles .02 taper (Denstply Maillefer, Ballaigues, Switzerland) from 10 to 40 to the working length with a filing motion.

Root canal preparations were performed by a single operator to avoid interoperator variation. All canal preparation techniques used in the study consist of seven files each. Files were used only one time and then they were discarded, and the preparation time did not exceed 10 minutes for each tooth. Apical patency was verified in all groups with a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland). Canals were irrigated with 1.5 mL of 5% sodium hypochlorite between each file with a Monojet syringe with a 30-G needle placed 3 mm short of the working length. Teeth were extracted 10 minutes later after ending canal preparation with conventional methods without excessive injury to periodontal ligament. After extraction, a #10 K-file was placed into the canal until its tip protrudes from the foramen to corroborate apical patency and that all working lengths were at 0.5 mm from the foramen. Periodontal ligament samples were obtained from the apical 3 mm of the root with a periodontal curette, placed on an Eppendorf tube, snap frozen in liquid nitrogen, and kept at −70°C until use.

Radioimmunoassay

Periodontal ligament samples were defrosted without thermal shock, dried on a filter, and weighed on an analytic balance. Neuropeptides were extracted by adding 150 μL of 0.5 mol/L of acetic acid and double boiling in a thermostat bath for 30 minutes in accordance with previously reported protocols (19–22). SP and CGRP expression were determined by competition binding assays using human SP and human CGRP-radioimmunoassay (RIA) kits (RK-061-05 and RK-015-02; Phoenix Peptide Pharmaceutical, Belmont, CA). Two different RIA assays were conducted, one for each neuropeptide.

In both RIA assays, a total of 50 μL of each sample solution was incubated in polypropylene tubes at room temperature for 20 hours with 100 μL of primary antibody (for each neuropeptide) and 100 μL of different SP (or CGRP) concentrations (10 pg/mL-1,280 pg/mL). Then, 50 μL of 125I-SP (or 125I-CGRP) was added and left to incubate for another 24 hours. Bound fractions were precipitated by the addition of 100 μL of a secondary antibody (goat antirabbit immunoglobulin G serum), 100 μL of normal rabbit serum, and 500 μL 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°C. The supernatants were decanted, and pellet radioactivity was read on a Gamma Counter (Gamma Assay LS 5500; Beckman, Fullerton, CA). Standard 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 SP and CGRP present in every sample using the percentage of maximum binding (B/B0%) calculated for each unknown sample and reading across 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 SP and CGRP concentrations in picomoles per milligram of periodontal ligament. The mean standard deviation and maximum/minimum values are presented for each group. The Kruskal-Wallis test was performed to establish statistically significant