Assessment of Apical Expression of Alpha-2 Integrin, Heat Shock Protein, and Proinflammatory and Immunoregulatory Cytokines in Response to Endodontic Infection

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Abstract

Introduction: The purpose of this study was to examine alpha-2 integrin, molecular mediators, cytokines, and chemokines from cells in periapical interstitial fluid from root canal infections before and after the reduction of the bacterial load using a cleaning procedure. **Methods:** Subjects included 20 patients referred to the School of Dentistry at the Universidade Federal de Minas Gerais (Belo Horizonte, Minas Gerais, Brazil). Clinical samples were taken from teeth with pulp necrosis, and no patients had acute periapical symptoms at the time of the appointments. After cleaning and drying, 3 paper points were introduced into the root canal, passing passively through the root apex (2 mm) into the periapical tissues for 1 minute. The samples were collected immediately after root canal cleaning and 7 days later (restrained root canal bacterial load) to characterize those gene expressions using real-time polymerase chain reaction. **Results:** Significantly lower levels of tumor necrosis factor alpha, chemokine ligand 5 (CCL5), chemokine ligand 2/monocyte chemotactic protein 1 (CCL2/MCP-1), and interleukin (IL)-8 in teeth with restrained bacterial loads (second collection) compared with the first collection were observed ($P < .05$). Similarly, the messenger RNA expression of the integrins secreted phosphoprotein 1 (SSP1)/ostepontin and focal adhesion kinase (FAK) decreased in samples from the second collection ($P < .05$). The messenger RNA for the regulatory cytokine IL-10 was significant higher in samples from the second collection (day 7) compared with the first collection (day 0) ($P < .05$). Messenger RNA expression of IL-1β, IL-17A, interferon gamma, alpha-2 integrin, and Hsp47/SERPINH1 were similar at both time points ($P > .05$). **Conclusions:** These findings suggest that after reducing the root canal bacterial load a decrease in the inflammatory response took place in the periapical lesions. (*J Endod 2015;41:1085–1090*)

Key Words

Apical periodontitis, FAK, Hsp47/SERPINH1, integrin, ITGA2, SSP1/OPN

Apical periodontitis is an inflammatory disease of periradicular tissues caused by the host immune response to root canal infection characterized by localized inflammation concomitant with bone resorption. The pathogenesis of periapical disease involves degradation of extracellular matrix components, including laminin, fibronectin, collagen, and proteoglycans (1).

Lymphocytes are the most prevalent cells in the periapical inflammatory infiltrate (2). These cells are associated with the production of cytokines and chemokines that restrain the root canal infection (3) but also lead to alveolar bone destruction.

The antigen-presenting cells, especially dendritic cells and macrophage, are responsible, at least, for the polarization of 4 different Th subsets (4). The inflammatory response is related to the Th1 subset, which produces cytokines like interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and interleukin (IL)-1, which are involved in the progression, bone destruction, and remodeling of periapical lesions (5). Conversely, the healing process is related to the Th2 subset. The Th17 subset may play a role in exacerbating inflammation by stimulating the secretion of proinflammatory mediators, such as IL-8, TNF-α, and IL-6 (6). Regulatory T (Treg) cells maintain normal homeostasis and reduce the overactivity of the Th1, Th2, and Th17 response. IL-10 exhibits strong anti-inflammatory properties and is produced by Th1, Th17, Th2, and Treg cells (7).

Cleaning and shaping procedures play a role in the reduction of root canal microbiota and its by-products, allowing the proper healing of apical tissues (8). This process is tightly regulated through cell-cell interactions and cell-matrix signaling and promoted by integrins, a variety of cytokines and growth factors, and cell mitosis and apoptosis (9).

Integrins are a large family of heterodimeric membrane glycoproteins involved in cellular processes mediated by cell-cell and cell–extracellular matrix interactions. Alpha-2 integrin mediates the induction of collagenase Matrix metalloproteinase-1 (MMP-1) in fibroblasts within collagen gels (10).

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Heat shock proteins (HSPs) exert a protective role against harmful environmental conditions and pathogens. They are characteristically induced by stress signals such as elevated temperature, infectious agents, and inflammatory mediators participating in the folding and translocation of polypeptides across the cell membrane (11). Hsp47 is the major collagen-binding heat-inducible glycoprotein expressed in fibroblasts (12).

The aim of this study was to quantitatively assay the messenger RNA (mRNA) expression of the alpha-2 integrin (ITGA2), heat shock protein 47 (Hsp47/SERPINH1), osteopontin (OPN)/SSP1, focal adhesion kinase FAK/PTK2, TNF-α, IFN-γ, IL-1β, IL-17, IL-10, CCL2/MCP-1, CCL5, and IL-8 in samples collected from interstitial fluid adjacent to human root canal infections. In addition, we detected the same integrins and cytokines 7 days later after the root canal cleaning procedures when root canal bacterial load was strongly reduced, providing conditions for healing process. Finally, we statistically compared and analyzed both sets of data.

Materials and Methods

Study Participants

Twenty patients referred to the Dental School at the Federal University of Minas Gerais, Belo Horizonte, Brazil, to receive endodontic care between the ages of 18 and 80 years old were recruited for this study. The exclusion criterion for this study was anti-inflammatory and antibiotic therapy up to 3 months before starting endodontic therapy. All participants signed the informed consent formulary. This study was approved by the Ethics Committee of the Federal University of Minas Gerais (CAAE 20383914.0.0000.5149).

Sample Collection

Clinical samples were taken from 20 teeth (single and multirooted) presenting pulp necrosis and apical periodontitis diagnosed by clinical and radiographic analyses and pulp sensibility tests. All selected patients failed to present acute periapical symptoms. Teeth were isolated by using a rubber dam followed by complete asepsis of the isolated crown with 30% hydrogen peroxide followed by 5% iodine and inactivation with 5% sodium thiosulfate as proposed by Möller (13). Cleaning and shaping of the root canals were completed by using ProTaper nickel-titanium files (Dentsply Maillefer, Ballaigues, Switzerland) in conjunction with 5.2% sodium hypochlorite. Collections were performed as previously described (14). Briefly, the samples were collected using paper points immediately after root canal cleaning to characterize the mRNA expression profiles of cytokines, chemokines, and integrins. After cleaning and drying, 3 paper points were introduced into the root canal through the root apex (2 mm) into the periapical tissues for 1 minute (day 0). The 4-mm tip of the paper points were dropped into a microcentrifuge tube, and the samples were stored at −70°C. By using this procedure, RNA was collected from the periapical interstitial fluid. No endodontic dressing was inserted into root canals. The coronal accesses of the teeth were restored with eugenol-based cement. Seven days later (day 7), the teeth were reopened, and the periapical interstitial fluid was sampled again to characterize the expressions of cytokines and integrins in teeth after instrumentation. In teeth with multiple canals, the first (day 0) and second (day 7) samples were collected from the same canal. No clinical signs or symptoms were present at the time of the second collection, and root canals were filled using the lateral condensation technique.

Sample Preparation

Total RNA was extracted from each sample with TRIzol reagent (GIBCO/BRL Laboratories, Grand Island, NY). Briefly, chloroform was added to the tubes containing the paper points, and the mixture was centrifuged at 12,000g at 4°C for 15 minutes. The aqueous phase was collected, and RNA was precipitated by isopropanol; 75% cold ethanol was added, and samples were centrifuged again at 12,000*g* at 4°C for 15 minutes. Ethanol was discarded, and samples were dried, dissolved in RNase-free water, and then incubated at 55°C for 10 minutes. The RNA was then stored at −70°C.

Real-time Polymerase Chain Reaction

Complementary DNA was synthesized by using 1 μg RNA through a reverse-transcription reaction as described by Barbosa Silva et al (15). Polymerase chain reaction (PCR) was performed under standard conditions: a holding stage of 95°C (10 minutes) and a cycling stage with 40 cycles of 95°C (15 seconds) followed by 60°C (1 minute) and a melt curve stage of 95°C (15 seconds), 60°C (1 minute), and 95°C (15 seconds). The primer sequences used for quantitative PCR analysis of IFN-γ, TNF-α, IL-1β, IL-17, IL-10, CCL2/MCP-1, IL-8, CCL5, ITGA2, Hsp47, OPN, FAK, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression are shown in Table 1. The sequences of human primers were designed by using PRIMEREXPRESS software (Applied Biosystems, Foster City, CA) based on nucleotide sequences available in the GenBank database. The real-time PCR assay was performed by using the StepOne Real-time PCR System (Applied Biosystems). A Syber-Green detection system (Applied Biosystems) was used to assay primer amplification. GAPDH was used as a housekeeping gene for normalization. All samples were run in duplicate. Sequence Detection System Software version v 2.4.1 (Applied Biosystems) was used to analyze data after amplification. Results were obtained as threshold cycle (C_T) values. Expression levels were calculated by using the comparative $2^{-\Delta\Delta CT}$ method (16). Briefly, with the $2^{-\Delta\Delta CT}$ method, the data are presented as the fold change in gene expression normalized to an

TABLE 1. Primer Sequences

Gene	Sense and antisense	Mt (°C)	bp
GAPDH	5′-GCA CCA CCA ACT GCT TAG CA-3′ 5′-TGG CAG TGA TGG CAT GGA GGA-3′	80	96
TNF-α	5′-TTC TGG CTC AAA AAG AGA ATT G-3′ 5′-TGG TGG TCT TGT TGC TTA AGG-3′	76	73
IL-1β	5′-TGG CAG AAA GGG AAC AGA A-3′ 5′-ACA ACA GGA AAG TCC AGG CTA-3′	73	59
IL-10	5′-GGT TGC CAA GCC TTG TCT GA-3′ 5′-TCC CCC AGG GAG TTC ACA T-3′	81	107
IL-17	5′-CAA TGA CCT GGA ATT ACC CAA-3′ 5′-TGA AGG CAT GTG AAA TCG AGA-3′	70	52
IFN-γ	5′-ACT GAC TTG AAT GTC CAA CGC A-3′ 5′-ATC TGA CTC CTT TTT CGC TTC CC-3′	61	101
ITGA2	5′-GCA GAT GGA CCA CAC TTT GA-3′ 5′-TGT CTG TGC CCT TTT CCT CT-3′	60	115
HSP47	5′-TGC TGA GCC CGG AAA CTC-3′ 5′-TTC AGG GCA GGC AGA ATG-3′	62	63
IL-8	5′-GAA TGG GTT TGC TAG AAT GTG ATA-3′ 5′-CAG ACT AGG GTT GCC AG ATT TAA C -3′	60	129
CCL5	5′-CGT GCC CAC ATC AAG GAG TA-3′ 5′-CAC ACA CTT GGC GGT TCT TTC-3′	80	91
OPN	5′-GCC GAG GTG ATA GTG TGG TT-3′ 5′-TGA GGT GAT GTC CTC GTC TG-3′	62	101
FAK	5′-CAA CAG GTG AAG AGC GAT TA-3′ 5′-CCA GTA TGA TCG CCG TAT TT-3′	58	99
CCL2	5′-AGG ACC ATT GTG GCC AAG GA-3′ 5′-CGG AGT TTG GGT TTG CTT GT-3′	81	93

bp, base pairs of amplicon size; CCL, chemokine ligand; FAK, focal adhesion kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Hsp47, heat shock protein 47; IFN-γ, interferon gamma; IL, interleukin; ITGA2, alpha-2 integrin; Mt, melting temperature; OPN, osteopontin; TNF-α, tumor necrosis factor alpha.