ORIGINAL ARTICLE

Detection of Tumor Necrosis Factor α in Normal and Inflamed Human Dental Pulp

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Background. The aim of this study was to determine concentrations of tumor necrosis factor-α (TNF-α) in normal, painful, and asymptomatic human dental pulps.

Methods. Pulp tissues were obtained from three groups of teeth, including healthy teeth, asymptomatic teeth with caries and/or large restoration, and symptomatic teeth with clinical diagnosis of irreversible pulpitis. Pulpal tissues were collected, prepared, and analyzed for TNF-α concentration by ELISA technique.

Results. Nonparametric Kruskal-Wallis test revealed significant differences between TNF-α concentration in normal samples (64.01 ± 53.12 pg/g) and irreversible symptomatic pulpal tissue (1,962.99 ± 1,288.75 pg/g), between irreversible symptomatic and asymptomatic (1,120.09 ± 649.72 pg/g), and between normal and irreversible asymptomatic pulpal tissue (p = 0.000).

Conclusions. TNF-α may be an objective marker for determining extent of pulpal inflammation associated with irreversible pulpitis. © 2002 IMSS. Published by Elsevier Science Inc.

Key Words: Irreversible asymptomatic pulpitis, Irreversible symptomatic pulpitis, TNF-α.

Introduction

Cytokines are intercellular regulatory proteins that mediate multiple immunologic and non-immunologic biological function (1). The term interleukin is used to describe a cytokine responsible for intercellular communication between leukocytes. Association or correlation with systemic inflammatory disease or tissue pathosis has been well documented (2).

Tumor necrosis factor (TNF) is a cytokine initially identified as a cause of hemorrhagic necrosis in certain tumors and was later shown to be the same molecule as cachectin, a serum product earlier known as a mediator of wasting in chronic disease.

TNF is secreted by both monocytes and T cells and exists in two forms, TNF-α and TNF-β, which are structurally and functionally related (3). TNF-α is a product of macrophages, while TNF-β (lymphotoxin) is a product of activated leukocytes. Both molecules are powerful modulators of bone resorption and inhibitors of collagen production (3). Produced in great abundance, TNF is capable of causing chronic wasting syndrome and anorexia and ensuring weight loss, cachexia, and lethal Gram-negative shock.

In addition to these diverse bioactivities, TNF is the only molecule other than IL-1 that is presently known to have osteoclast-activating function (4). No direct evidence has been presented to show the presence of TNF-α in either normal or inflamed dental pulp, despite considerable interest in mechanisms and consequences of inflammation and pain in this tissue (5). The purpose of this study was to determine whether TNF-α could be detected in normal, symptomatic, and asymptomatic pulpal tissues classified according to clinical symptoms.
Materials and Methods

Sample collection. A total of 60 human teeth were included in this study. The teeth were divided into three groups. Group 1 contained normal pulp tissue collected from 20 teeth. Teeth were placed in this group based on the following criteria: verbal history confirming no history of pulpal pain, and clinical and radiographic examination assuring that these teeth had no caries, restorations, or periodontal disease (6). Teeth in this group included impacted ectopically erupted third molars and bicusps extracted due to orthodontic considerations (7).

Group 2 consisted of pulp tissue collected from 20 teeth with clinical diagnosis of irreversible asymptomatic pulpitis (IAP). Teeth were placed into this group based on the following criteria: verbal history confirming no history of pulpal pain, and clinical and radiographic examination determining presence of caries and/or large amalgam or composite restorations, no signs of periapical pashosis, no sensitivity to percussion or palpation, and teeth testing sensitive to ice.

Group 3 consisted of pulp tissue collected from 20 teeth with clinical diagnosis of irreversible symptomatic pulpitis (ISP). Diagnosis was made on the following criteria: teeth were currently causing spontaneous pain or had a recent history of causing severe, prolonged pain to thermal stimuli, and clinical and radiographic examination determining presence of near or actual pulp exposure, absence of periapical lesions, sensitivity to percussion, no palpation sensitivity, and vitality test on cold showing prolonged pain. Teeth in groups 2 and 3 were extracted because the patient refused endodontic treatment. Extraction was done under local anesthesia (1.8 mL Xylestesin-Forte, ESPE, Seefeld, Germany) without complications.

Immediately following extraction, a longitudinal groove was placed in the crown of the tooth under water coolant with a #557 carbide bur and high speed. Teeth were fractured by twisting a #3 elevator in the prepared groove. Following tooth fracture, pulpal tissue was gently removed. Tissue was transported on ice to the laboratory and stored at −80°C until prepared for enzyme-linked immunosorbent assay (ELISA, Sigma Immuno Chemicals TNF-α kit, stock no. CKH-200 A, Sigma Chemical Co., St. Louis, MO, USA) procedures (8). All experimental samples were subjected to standardized collection, storage, and processing procedures.

Enzyme-linked immunosorbent assay (ELISA). Frozen pulpal samples were thawed for 30 min and weighed. A total of 100 μL of phosphate-buffered saline (PBS), pH 7.0 was added to each specimen and tissue was crushed with a small glass rod to elute TNF-α from pulpal tissues.

Elutions were performed at 4°C over a 30-min period with mixing before centrifugation for 2 min at 9,880 g. Standard of human tumor necrosis factor-α (TNF-α) ELISA Kit (Sigma Immuno Chemicals TNF-α kit, stock no. CKH-200 A) and eluted pulpal samples were applied to 96-well microtiter plates. All tests were performed in duplicate, repeated three times, and assayed according to ELISA procedure as follows: 1) 50 μL of assay diluent 1F (D-1550) was added to each well; 2) 200 μL of sample was added per well, covered with the provided plate cover (C-5697), and incubated for 1 h in the shaker (Redrotor Hoefer Pharmacia Biotech, Inc., San Francisco, CA, USA); 3) 200 μL of TNF-α conjugate (T-8549) was added, covered with a new plate cover, and incubated 1 h in the shaker; 4) substrate solution was prepared by mixing equal volumes of color reagent A (R-6517) and color reagent B(R-6642) together within 15 min of use; 5) 200 μL of substrate solution was added to each well and incubated 20 min at room temperature; 6) 50 μL of stop solution (S-0416) was added to each well and gently mixed, and 7) optical densities (OD) of individual wells were obtained spectrophotometrically (450 nm) within 30 min.

TNF-α concentration calculations. With use of standard dilution concentration and OD, a standard curve was prepared for each plate, and amount of TNF-α (units/μL) was calculated by linear regression analysis for each pulpal sample. Amount of TNF-α units (pg) per mass of tissue specimen (g) was determined using the following formulas: a) pulp weight (g/0.100 mL) = pulp concentration (g/mL), and b) TNF-α concentration (pg/mL)pulp concentration (g/mL) = TNF-α (pg/pulp) (g).

Statistical analysis. TNF-α data in pg/g of tissue were statistically analyzed using nonparametric Kruskal-Wallis test for the three groups classified by clinical diagnosis ($\chi^2 = 29,354.10$, df = 2, $p = 0.000$).

Results

The present study demonstrated presence of TNF-α in all vital pulp samples. During preparation for ELISA analysis, five samples were lost (two healthy, two irreversible asymptomatic pulps, and one irreversible symptomatic).

Differences in concentration of TNF-α of healthy pulp tissue (64.01 ± 53.12 pg/g), asymptomatic pulpitis (1,120.09 ± 649.72 pg/g), and irreversible symptomatic (1,962.99 ± 1,288.75 pg/g) were statistically significant ($p = 0.000$). There was also statistically significant difference between the samples of irreversible symptomatic and asymptomatic pulpitis. ELISA data for TNF-α were categorized according to clinical diagnosis and nonparametric Kruskal-Wallis test was used ($\chi^2 = 29,354.10$; df = 2; $p = 0.000$) (refer to graphic representation data in Figure 1).

Discussion

Biologic pulpal response to injury and events that control these processes are not completely understood (1). Diagnos-
tic assessment of biologic markers may improve validity of predicting or treating inflammatory pulpal disease.

With the exception of its unique anatomic position, dental pulp responds to bacterial infection in the same manner as other connective tissue in the organism. Extent of pulpal response to bacterial irritation varies from slight tissue inflammation to complete necrosis (9).

Several biologic molecules have been identified in inflamed pulpal tissues and found present in greater concentrations than those reported for healthy pulpal tissues. These substances are chemotactic for inflammatory cells such as polymorphonuclears and macrophages (1).

TNF-α protein has a particularly potent effect on neutrophil leukocytes and induces chemotaxis and activation of neutrophils. Under the influence of TNF-α, dilatation and increased permeability of blood vessels occur, causing extravasation of leukocytes from blood into the infected area. Cytokines activate inflammatory cells in intracellular areas, causing increased phagocytosis, release of toxic modulators/agents, and possible elimination of bacteria (10).

This study provides relevant information concerning pathogenesis of pulpal inflammatory disease. Highest concentrations of TNF-α were detected in irreversible symptomatic pulps. As irreversible symptomatic pulpal inflammation progressed, TNF-α concentration decreased. It may be speculated that decrease in TNF-α concentration represents a point at which tissue is in late phase of irreversible inflammation, progressing toward total tissue necrosis. Similar findings have been reported by Tani-Ishii et al. (11) who showed that the number of TNFα-expressing cells increased five times—from zero to second day, and 10 times from second to fourth day—and thereafter decreased until seventh day of inflammation induced in rats. A study by Kjeldsen et al. (3) using ELISA technique has also shown significantly higher concentration of TNF-α in crevicular fluid of patients with chronic adult periodontitis in comparison to healthy subjects. It is likely that chronic periodontal infection may evoke an immune response that may result in production of slightly higher levels of TNF-α. Therefore, it was suggested that this cytokine may be a marker of early inflammation (3). Elevated TNF-α levels detected in periapical tissue exudates of human apical periodontitis may also have far-reaching systemic consequences (12).

Because TNF-α is a cytokine with numerous inflammatory effects in chronic disease pathogenesis including pulpitis and periodontitis, detection of this mediator may be of great importance to differentiate samples of irreversible symptomatic and asymptomatic pulps. Therefore, further investigations are warranted to elucidate the role this important mediator may play in oral infections.

In conclusion, TNF-α can be found in all vital human pulpal tissues. Highest concentrations of this protein were found in irreversible symptomatic pulps and slightly less in irreversible asymptomatic pulps, while lowest TNF-α concentration was found in healthy samples. Results indicate that TNF-α may be an objective marker for laboratory determination of extent of pulpal inflammation.

References