International Endodontic Journal

doi:10.1111/j.1365-2591.2009.01567.x

The upregulation of oncostatin M in inflamed human dental pulps

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Abstract

Huang F-M, Tsai C-H, Yang S-F, Chang Y-C. The upregulation of oncostatin M in inflamed human dental pulps. *International Endodontic Journal*, **42**, 627–631, 2009.

Aim To compare oncostatin M (OSM) expression in clinically healthy and inflamed specimened human pulp tissue.

Methodology Thirty pulpal tissue specimens (15 clinically healthy and 15 inflamed) were obtained from extracted third molars with informed consent from patients. The levels of OSM were compared between clinically healthy pulp and inflamed pulp tissues using the semi-quantitative reverse-transcriptase polymerase chain reaction. In addition, immunohistochemistry was used to identify the *in situ* localization of OSM expression in pulp specimens. For testing of differences in the OSM between the clinically healthy and inflamed human dental pulps, the Wilcoxon–Mann–Whitney rank sum test was applied. Differences in OSM expression between tissue with low and high levels of inflammation were subsequently analysed by Fisher's exact test.

Results Inflamed pulps exhibited significantly higher OSM mRNA gene expression than clinically healthy pulps ($P < 0.05$). Immunohistochemistry demonstrated that OSM expression was significantly higher in inflamed than clinically healthy pulps ($P < 0.05$). OSM staining was detected in odontoblasts, fibroblasts, inflammatory infiltrates and endothelial cells.

Conclusions Oncostatin M expression was elevated in inflamed pulp tissue. OSM is potentially involved in the disease process of pulpal inflammation.

Keywords: inflammation, oncostatin M, pulp.

Received 21 September 2008; accepted 19 February 2009

Introduction

Dental pulp is a loose mesenchymal tissue almost entirely enclosed in dentine. The most common pulp injury occurs as a result of the penetration of bacteria or their surface components into the tissue through dentine resulting from either caries or accidental exposure. The end result of the inflammatory process is a necrotic pulp devoid of viable tissue. Like any other types of inflammation, pulpal inflammation is associated with tissue degradation.

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Oncostatin M (OSM), a pleiotropic glycoprotein cytokine that was originally purified from conditioned media of phorbol ester-stimulated U937 (histocytic lymphoma) cells, is related structurally and functionally to the subfamily of haematopoietic and neurotrophic cytokines known as the interleukin (IL)-6 cytokine family (Zarling *et al.* 1986). OSM, a 28 kDa glycoprotein produced pre-dominately by activated T-lymphocytes and endotoxin stimulated macrophages, is a member of the IL-6 family, which comprises IL-6, IL-11, OSM, ciliary neurotrophic factor and leukemia inhibitory factor (Heinrich *et al.* 2003). In addition, polymorphonuclear neutrophils have been described recently to be potent cellular sources of OSM biosynthesis and release under inflammatory conditions (Grenier *et al.* 1999, Hurst *et al.* 2002). OSM participates in growth regulation, differentiation, gene expression and also contributes to inflammation and

tissue remodeling processes (Heinrich *et al.* 2003, Tanaka & Miyajima 2003). Increased expression of OSM has been detected in inflammatory diseases such as chronic periodontitis (Lin *et al.* 2005, Lu *et al.* 2006) and epithelialized apical periodontitis lesions (Tsai *et al.* 2008). These suggest that OSM may directly cause the deterioration associated with inflammatory process.

In this study, it was hypothesized that OSM may play a role in the pathogenesis of pulpal inflammation. To date, immunohistochemical localization and further molecular biological studies of inflamed dental pulps are indicated to elucidate further the complex nature of OSM in inflamed human dental pulps. In this study, levels of OSM expression in clinically healthy and inflamed human pulps were evaluated by using semi-quantitative reverse-transcriptase polymerase chain reaction (RT-PCR). In addition, immunohistochemistry was used to determine the *in situ* localization of OSM expression in dental pulps.

Materials and methods

Sample collection

Thirty pulpal tissue specimens (15 clinically healthy and 15 inflamed pulps) were obtained from extracted third molars with informed consent from patients at the Department of Oral Surgery, Chung Shan Medical University Hospital, Taichung, Taiwan. Medical histories of all patients were non-contributory and patients were not receiving any long-term anti-inflammatory medication. The inflamed pulps were obtained from teeth with carious pulp exposure showing spontaneous pain and/or lingering pain in response to cold and/or heat stimulus. Asymptomatic pulps from non-carious teeth that had no radiographical evidence of caries or periapical pathosis were used as normal controls.

Preparation of pulpal tissue specimens

Immediately after extraction, teeth were longitudinally grooved with a fissure bur and then split in half. The dental pulps were extracted carefully using a spoon excavator. For RT-PCR assay, pulp tissue samples were manually homogenized in saline in individual Eppendorf tubes. For immunohistochemistry, tissues were fixed with 10% buffered formalin overnight, the specimens were dehydrated in an ascending series of graded alcohols and embedded in paraffin.

Reverse-transcriptase polymerase chain reaction

Total RNA was prepared using TRIzol reagent (Gibco Laboratories, Grand Island, NY, USA) and following the manufacturer's instructions. Single-stranded DNA was synthesized from RNA in a 15 μL reaction mixture containing 100 ng random hexamer and 200 units of Moloney murine leukemia virus reverse transcriptase (Gibco Laboratories). The reaction mixture was diluted with 20 μL of water and 3 μL of the diluted reaction mixture was used for the PCR. PCR reaction mixture contains 10 pmol L^{-1} of forward and reverse primers and 2 units of Taq DNA polymerase. Amplification was performed at 25 cycles for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and 30 cycles for OSM in a thermal cycler. Each cycle consisted of 1 min of denaturation at 94 °C, 1 min of annealing at 57 °C, and 1 min of extension at 72 °C. The sequences of primers used were as follows (Suda *et al.* 2002):

GAPDH	sense: CGGAGTCAACGGATTTGGTCGTAT
	antisense: AGCCTTCTCCATGGTTGGTGAAGAC
OSM	sense: CGTATCCAAGGCCTGGATGTT
	antisense: GCCCTCCAGCTTGCGCTGAAA

The PCR products were analysed by agarose gel electrophoresis. When the tissues were probed for OSM mRNA production by RT-PCR, a 393 bp band for OSM were noted. These bands were consistent with the size as designed by primers. When the band densities were measured and compared with the density of the band obtained for the housekeeping gene GAPDH, relative proportions of mRNA synthesis could be determined within each experiment. The intensity of each band after normalization with GAPDH mRNA was quantified from photographed gels with a densitometer (Alpha-Imager 2000; Alpha Innotech, San Leandro, CA, USA). Each densitometric value was expressed as the mean ± SD from triplicate experiments. For testing of differences in the OSM between the clinically healthy and inflamed human dental pulps, the Wilcoxon–Mann–Whitney rank sum test was applied. A P-value of <0.05 was considered to be statistically significant.

Immunohistochemistry

Serial 5 μm thick sections from each specimen were stained with the monoclonal anti-OSM antibody [rabbit anti-human, OSM (N-1): sc-129; Santa Cruz Biotechnology, CA, USA] (1 : 50 dilution) using a standard avidin–biotin peroxidase complex method as described

previously (Tsai *et al.* 2005, 2008). Diaminobenzidine (Zymed, South San Francisco, CA, USA) was then used as the substrate for localizing the antibody binding. The preparations were counterstained with haematoxylin, mounted with Permount and examined by light microscopy. To demonstrate the specificity of the staining, negative controls were included in which the primary antibody was replaced with phosphate-buffered saline.

Processed immunohistochemically for OSM expression, sections graded as 'low' were represented by positive stained cells <50%; sections graded 'high' exhibited positive stained cells over 50% on three sections/tissue at 400× magnification. Fisher's exact test was applied for the statistical analysis of the results. A *P*-value of <0.05 was considered to be statistically significant.

Results

Reverse-transcriptase polymerase chain reaction assay for OSM were used to compare the mRNA levels between clinically healthy pulp and inflamed pulp tissues. Inflamed pulp specimens exhibited significantly higher OSM mRNA expression than clinically healthy pulp tissues (Fig. 1). The average of OSM mRNA from inflamed pulps was elevated approximately 2.36 fold compared with clinically healthy pulps ($P < 0.05$) (Fig. 2).

Oncostatin M staining was stronger in inflamed pulps than in clinically healthy pulps. In clinically healthy pulp specimens, OSM staining was scarcely detected and almost totally limited to the endothelium (Fig. 3a). All of the inflamed pulps revealed the presence of OSM positive cells. OSM expression was observed mainly in the cytoplasm of odontoblasts, fibroblasts, inflammatory cells and endothelial cells (Fig. 3b).

From the results of immunohistochemistry, two cases of clinical healthy pulps (30%) and 12 cases

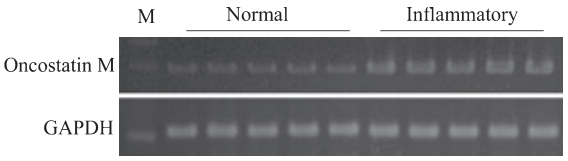

Figure 1 Comparison of the Oncostatin M (OSM) mRNA gene level from clinically healthy pulps and inflamed pulps using reverse-transcriptase polymerase chain reaction assay. GAPDH gene was performed in order to monitor equal RNA loading. Inflamed pulps are significantly upregulated OSM mRNA gene expression than clinically healthy pulps.

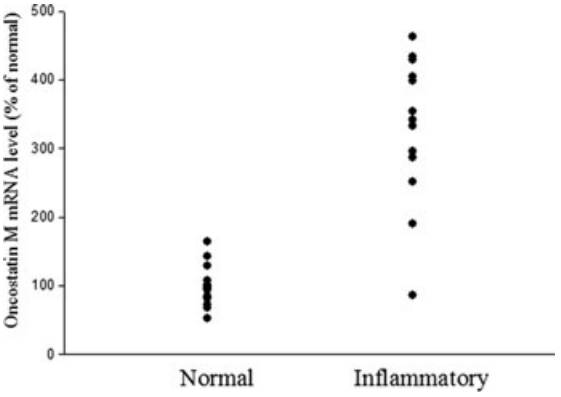

Figure 2 Densitometric analysis of OSM bands, the intensity of Oncostatin M mRNA from inflamed dental pulps was elevated about 2.36 fold compared with clinical healthy pulps ($P < 0.05$).

(80%) of inflamed dental pulps exhibited high OSM expression. A total of 13 cases of clinical healthy pulps (86.7%) and three cases (20%) of inflamed dental pulps exhibited low OSM expression. Differences in OSM expression between tissues with high or low intensity were subsequently analysed using Fisher's exact test. A significantly greater OSM expression was noted in inflamed human dental pulps than those of clinically healthy pulps ($P < 0.01$).

Discussion

Oncostatin M is a pleiomorphic cytokine that belongs to IL-6 cytokine family. It plays an important role in the process of inflammatory reactions. It has been demonstrated that human pulp tissues can express OSM mRNA gene at the transcription level. In addition, levels of OSM in inflamed pulps were significantly higher than those recorded in clinically healthy pulps. In addition, histological evidence is presented, which supports the finding that OSM expression is upregulated in inflamed dental pulps. Strong immunostaining for OSM was detected in odontoblasts, fibroblasts and inflammatory cell infiltrates. These observation are in agreement with the increased levels of OSM expression in inflamed gingival tissues (Lin *et al.* 2005, Lu *et al.* 2006) and epithelialized apical periodontitis lesions (Tsai *et al.* 2008). OSM may be involved in the disease process of pulpal inflammation.

Polymerase chain reaction is a rapid and powerful technique for the *in vitro* amplification of DNA (Gibbs 1990). In this study, GAPDH was used as a housekeeping gene to confirm the homogeneity of RNA

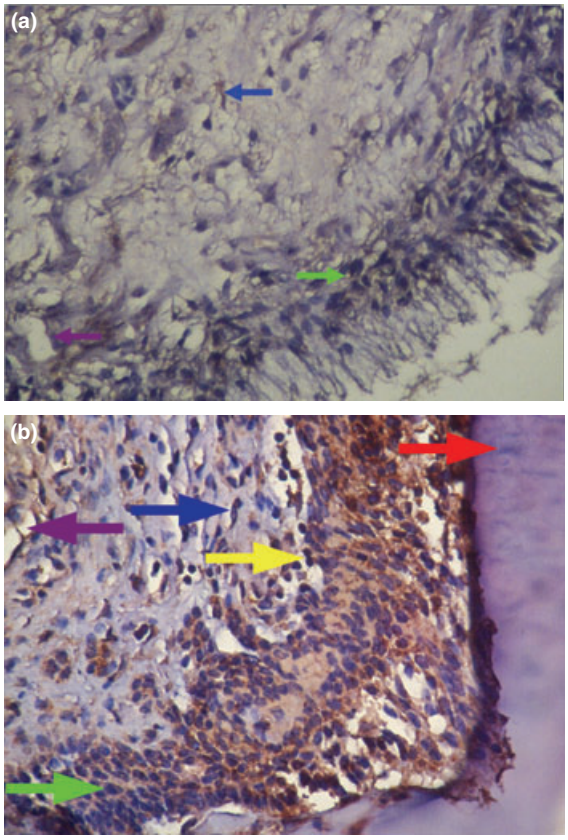

Figure 3 (a) Very faint immunoreactivity of Oncostatin M (OSM) was observed in clinically healthy human pulp and almost totally limited to the endothelium. (200×) (b) Immunolocalization of OSM in an inflamed pulp specimen. OSM stain was detected in the fibroblasts, odontoblasts, inflammatory infiltrates, and endothelial cells. (×400) Red arrow, dentine; green arrow, odontoblast; yellow arrow, inflammatory cell; blue arrow, fibroblasts; purple arrow, endothelial cells.

loading in individual lanes and GAPDH expression levels showed similar patterns. The reactions for GAPDH mRNA were not saturated. However, PCR has to be considered be a semi-quantitative procedure.

Somewhat oversimplified, quantitative power can be defined as being the level of reproducibility, precision, and accuracy achievable when quantitating a specified amount of nucleic acids. The key factors that influence the quantitative ability of a PCR procedure are the following (Ferre 1992): (i) Optimization of the amplification step. (ii) Hot start and new tricks. (iii) Linear range of amplification. If done properly, PCR becomes a powerful quantitative tool. Otherwise, only real-time-PCR can produce quantitative data.

Modur *et al.* (1997) have demonstrated that OSM fulfills the Koch's postulates as an inflammatory mediator. The activation of exogenous destructive pathways may be mediated by an immune response resulting in the expression of degradative cellular phenotypes between both immigrant and residual cell populations. Recently, it has also reported that bacterial lipopolysaccharide can induce OSM in dentritic cells (Suda *et al.* 2002) and astrocytes (Repovic *et al.* 2003). Previous studies also demonstrated that inflammatory cytokines IL-1α, TNF- α, and IL-6 were present in surgical pulp exposure specimens (Tani-Ishii *et al.* 1995, Barkhordar *et al.* 1999). OSM expression in inflamed pulps may be induced either directly by bacteria or indirectly by inflammatory cytokines generated by resident cells. Thus, these cells may play an important role in the pathogenesis of pulpal inflammation by controlling the synthesis of proinflammatory cytokines and OSM.

In addition, OSM alone may stimulate the production of IL-6, or act synergistically with IL-6 to elevate the production of MMP or IL-6 production respectively (Brown *et al.* 1991, Manicourt *et al.* 2000). This suggests that OSM may directly cause the deterioration associated with inflammatory processes or it may act in concert with other cytokines to amplify IL-6 production or contribute to the inflammatory cycle. Moreover, MMPs were found to play an important role of the pathogenesis in the pulpal inflammation (Chang *et al.* 2001, 2002, Shim *et al.* 2002, Huang *et al.* 2004, Tsai *et al.* 2005). Thus, the interaction between OSM and MMP in the pathogenesis of pulpal inflammation needs further investigation.

Oncostatin M could be detected in inflamed cell areas in inflamed pulp tissue which suggests that OSM stored in the cytosol of odontoblasts, fibroblasts, inflammatory, and endothelial cells may represent a reservoir of OSM activity that can be released at certain stages of the inflammatory reaction. It appears that the presence of such intracellular storage permitted the immunohistochemical detection of OSM in pulp tissues. The study has shown that there is good evidence to suspect that OSM plays a significant role in the tissue destruction associated with pulpal inflammation.

Biological activities of OSM are mediated through binding to its specific OSM receptor subunit b (OSMRb), which belongs to the signal transducing receptors for IL-6 type cytokines (Heinrich *et al.* 2003). Cloning and expression of the OSMRb revealed that OSM restrictively transduces signals through its specific receptor complex composed of gp130 and OSMRb (Lindberg

et al. 1998, Tanaka *et al.* 1999). However, more detailed studies should be undertaken to clarify the role of OSM as well as OSMRb in the pathogenesis of pulpal inflammation.

Conclusion

In this study, inflamed pulps exhibited significantly higher OSM mRNA gene expression than clinically healthy pulps. OSM staining was stronger in inflamed pulps than in clinically healthy pulps. OSM expression was observed mainly in the cytoplasm of odontoblasts, fibroblasts, inflammatory cells and endothelial cells. OSM expression was elevated in inflamed pulp tissue.

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