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## The Adjunctive Effect of Tenoxicam During Non-Surgical Periodontal Treatment on Clinical Parameters and Gingival Crevicular Fluid Levels of MMP-8 and TNF- $\alpha$ in Patients with Chronic Periodontitis – Randomized, Double-Blind Clinical Trial

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### Abstract

**Background.** Matrix metalloproteinases (MMPs) and cytokines play a role of extracellular matrix degradation and remodelling, and are significantly involved in the course of periodontal disease.

**Objectives.** The purpose of this study was to evaluate the adjunctive effect of administering an oxycam non-steroidal anti-inflammatory drug (NSAID), tenoxicam, during non-surgical (phase I) periodontal treatment on clinical parameters and gingival crevicular fluid (GCF) levels of MMP-8 and TNF- $\alpha$  in subjects with chronic periodontitis.

**Material and Methods.** A total of 32 subjects with chronic periodontitis were randomized into two groups: 1) phase I periodontal treatment + NSAID and 2) phase I periodontal treatment + placebo. Phase I periodontal therapy consisted of scaling and root planning (SRP), which was provided by a single therapist masked with respect to group assignment. Patients in group 1 received a systemic NSAID (20 mg tenoxicam tablet once daily for 10 days). Clinical measures and GCF samples were obtained immediately prior to periodontal treatment and 30 days afterwards from all subjects. Clinical measures included a plaque index, gingival index, gingival bleeding time index, probing depth, and clinical attachment level. The MMP-8 and TNF- $\alpha$  levels in the GCF were assayed using an enzyme-linked immunosorbent assay.

**Results.** With the exception of clinical attachment level, all clinical measures showed a significant ( $p < 0.05$ ) improvement following non-surgical treatment in both the NSAID and placebo groups. A significant decrease in MMP-8 levels ( $p < 0.05$ ) was observed at post-treatment in the NSAID group but not in the placebo group ( $p > 0.05$ ). Treatment exhibited no effect on TNF- $\alpha$  levels ( $p > 0.05$ ). There was also no statistically significant difference in clinical measurements after treatment between the two groups ( $p > 0.05$ ). Moreover, the post treatment MMP-8 level in group 1 was statistically significant higher than the placebo group ( $p < 0.05$ ).

**Conclusions.** The adjunctive administration of tenoxicam during phase I periodontal treatment decreases MMP-8 levels in gingival crevicular fluid in patients with chronic periodontitis. But no benefits were observed (Adv Clin Exp Med 2014, 23, 4, 559–565).

**Key words:** MMP-8, TNF- $\alpha$ , chronic periodontitis, anti-inflammatory drug.

Periodontitis is characterized by chronic infection, inflammation, and destruction of the supporting periodontal tissues [1]. Matrix metalloproteinases (MMPs) are a family of important enzymes that remodel and degrade connective tissue in pathological tissue turnover [2–4]. Polymorphonuclear leukocytes

(PMNs) and macrophages synthesize a broad spectrum of matrix-degrading proteases, including MMPs, in periodontal diseases [3, 5, 6]. PMNs, therefore, play an important role in the MMP-mediated breakdown of periodontal tissue [7, 8]. MMP-8 is a principle PMN-derived collagenase that is thought

to contribute to periodontitis [9]. MMP-8 is detected in GCF samples from sites of periodontitis and plays a key role in destroying periodontal supporting tissues during chronic periodontal disease [10, 11]. Several studies have shown the potential utility of MMP-8 as a marker of periodontal treatment effectiveness and for identifying patients at risk of continuing attachment loss [12–15].

Among the many inflammatory and immune mediators identified in GCF, cytokines have attracted considerable attention because of their potential role in inflammation-related destruction and repair of the periodontal tissues. Increased levels of several cytokines, including interleukin (IL)-1, IL-2, IL-6, IL-8, and tumor necrosis factor (TNF)- $\alpha$  have been observed in the GCF of patients with periodontal disease [16]. Considerable interest has focused on the potential for cytokines to serve as diagnostic or prognostic markers for periodontal disease activity and wound healing [17]. TNF- $\alpha$ , for instance, has an extremely broad spectrum of biological activity and plays a central role in many inflammatory diseases [18]. Elevated levels of TNF- $\alpha$  have been found in the inflamed gingival tissue and GCF of patients with periodontal disease. TNF- $\alpha$  can induce/up-regulate MMP-8 expression via gingival fibroblasts [19].

Non-steroidal anti-inflammatory drugs (NSAIDs) are selective inhibitors of the cyclooxygenase pathway, thereby preventing the synthesis of prostaglandins, thromboxanes, and prostacycline. NSAIDs given as an adjunct to non-surgical periodontal treatment reduced gingival inflammation, rate of attachment loss, and alveolar bone loss [20]. Numerous studies have characterized the ability of NSAIDs to modify the host response in animal models and human trials [20, 21]. The recent reports that cyclooxygenase (COX) exist in at least two isoforms (COX-1 and COX-2) has led to the suggestion that the therapeutic benefits resulting from classical NSAID use are derived from a COX-2 blockade, whereas a concomitant COX-1 blockade by these drugs provokes side effects. Tenoxicam is one of the NSAIDs and had not studied before as an adjunct to periodontal treatment [22]. Therefore, the purpose of this study was to evaluate the adjunctive effect of administering tenoxicam, an oxycam NSAID, on clinical parameters and GCF levels of MMP-8 and TNF- $\alpha$  in subjects with chronic periodontitis.

## Material and Methods

### Patient Selection and Clinical Procedures

A total of 32 subjects were enrolled in the present randomized, double-blind, placebo-controlled

study. A power analysis was performed with  $\alpha = 0.05$ ,  $\beta = 0.10$  and  $(1-\beta) = 0.90$ , and the power was calculated as  $p = 0.90833$ . Additionally, the sample size was found to be sufficient. Thirty-two subjects with chronic periodontitis (CP) were randomly divided into two groups. Group 1 was comprised of 16 subjects (7 females and 9 males,  $40.9 \pm 8.2$  years of age, range 31 to 57 years) that underwent scaling and root planing and received a systemic NSAID (20 mg tenoxicam tablet, once daily for 10 days). Group 2 was comprised of 16 patients (7 females and 9 males,  $42.3 \pm 7.3$  years of age, range 31 to 59 years) that underwent scaling and root planing and received placebo tablets once daily for 10 days.

Chronic periodontitis patients were selected from those referred to the Department of Periodontology, Faculty of Dentistry, Cumhuriyet University. Informed consent was obtained from all subjects, and GCF sampling and clinical procedures were explained fully prior to the study. Periodontal disease status was determined by clinical examination and radiographs. The CP patients had at least 4 periodontal sites with probing depths of 4–6 mm and radiographic evidence of bone and attachment loss involving the maxillary anterior teeth. Exclusion criteria involved (1) the presence of systemic diseases where administration of NSAIDs would be contraindicated such as stomach or duodenal ulcer, (2) presence of systemic illness or conditions which affect oral tissues such as insulin dependent diabetes mellitus, (3) known hypersensitivity to NSAIDs, (4) pregnancy, (5) smoking, and (6) any antibiotic, systemic corticosteroid, or immunosuppressive drug use within the past six months. The study protocol was approved by the Ethics Committee, Faculty of Dentistry, Cumhuriyet University. Randomization of subjects with periodontitis was accomplished by an investigator not involved in the treatment, clinical assessment, or laboratory analysis of the samples. Randomization was ensured using a coin-toss. All other investigators, including the statistician, remained masked with respect to drug therapy until all the data had been collected and submitted for analysis. Clinical evaluations were based on the following parameters: plaque index (PI) [23], gingival index (GI) [24], probing depth (PD), gingival bleeding time index [25], and clinical attachment loss (CAL). Measurements were recorded by a calibrated examiner. All clinical parameters were measured with a Goldman/Fox Williams probe calibrated in millimeters, and clinical measurements were performed on six sites per tooth at the study site (mesio-buccal, midbuccal, disto-buccal, and the same for the oral side). At the first appointment, upper anterior teeth with a PD of 4–6 mm were selected as

study sites to ensure SRP standardization. The following day, GCF samples were obtained and baseline clinical scores were recorded from the selected sites. The same teeth were chosen for clinical recording and GCF sampling for all groups to maintain standardization. The subjects were instructed on how to perform daily plaque control and to take the drugs at the same time each day. Subjects did not take any other drugs during administration of tenoxicam (Drogsan, Ankara, Turkey) or placebo. Drug intake was started on the same day as initial therapy. SRP was performed by sharp sickles and Gracey and universal curets in the study sites under local anesthesia for 10–11 min per tooth. Oral hygiene instructions were reinforced after the therapy. Clinical measurements and GCF samples were obtained again at 30 day. Additionally, periodontal treatment was performed on the other teeth.

### GCF Sampling and Processing

The GCF samples were collected using paper (Oraflow, Plainview, NY, USA) strips. The sample sites (the upper 4 incisors) were gently air-dried and all supragingival plaque removed. The area was isolated carefully with cotton rolls and a saliva ejector was used to prevent saliva contamination. The paper strip was inserted into the pocket until slight resistance was felt and left in place for 30 s [26]. Care was taken to avoid mechanical injury of the gingival tissues. Strips contaminated by bleeding or exudate were discarded. Four samples per patient were placed into Eppendorf tubes and stored at  $-80^{\circ}\text{C}$  until analysis.

### MMP-8 and TNF- $\alpha$

The levels of MMP-8 and TNF- $\alpha$  in GCF samples were assayed using commercially available sandwich enzyme linked immunosorbent assay (ELISA) kits (RayBio, Norcross, GA, USA and Invitrogen, Carlsbad, CA, USA). All assay procedures were carried out according to the manufacturers' instructions, using human recombinant protein standards. First, the GCF samples were eluted from the strips using a centrifugal method [27]. Elution was carried out with the addition of 1000  $\mu\text{L}$  phosphate buffer solution (pH 7.4). Next, the eppendorf tubes containing the strips and buffer were centrifuged for 20 min at  $3,000 \times g$ . After centrifugation, the fluid remaining in the tubes was used to measure MMP-8 and TNF- $\alpha$  levels using ELISA. The amount of crevicular MMP-8 and TNF- $\alpha$  in each sample was determined by standard calibration curves. The tetramethylbenzidine (TMB) substrate solution for MMP-8 or stabilized chromogen for TNF- $\alpha$  was added to the wells and

the color reaction assessed using a reader set to a wavelength of 490 nm. Results were calculated based on ELISA concentration values and reported as total enzyme and cytokine amounts (picogram  $\pm$  SD) per sample.

## Statistical Analysis

Statistical analysis was performed using SPSS version 14.0 (Microsoft Corp, Chicago, IL, USA). A Wilcoxon test was used for evaluation of the differences before and after treatment of the 2 groups. A Mann-Whitney  $U$  test was used for evaluation of the differences between group 1 and group 2. The correlation between clinical indices and MMP-8 and TNF- $\alpha$  levels was analyzed with a Pearson's correlation test. Statistical significance was set at  $p < 0.05$ .

## Results

### Clinical Results

The PI, GI, and GBTI scores were significantly reduced in the tenoxicam and placebo groups after therapy compared to baseline values ( $p < 0.05$ ) (Table 1). CAL scores remained unchanged following treatment in each group ( $p > 0.05$ ). Moreover, post-therapy values for clinical measures in the two groups are shown in Table 1. There was no significant difference in clinical measurements between group 1 and 2 after treatment ( $p > 0.05$ ).

### Laboratory Results

The pre- and post-treatment GCF parameters in groups 1 and 2 are given in Table 1. Although a reduction was observed in the TNF- $\alpha$  values after treatment in both groups 1 and 2, this difference was not significant ( $p > 0.05$ ). The post-treatment level of MMP-8 was significantly lower in the tenoxicam group compared to baseline ( $P < 0.05$ ) but not for the placebo group ( $p > 0.05$ ) (Table 1). Moreover, the post-treatment MMP-8 level in group 1 was statistically significantly higher than in the placebo group ( $p < 0.05$ ) (Table 1).

In addition, in both groups, a significant correlation was seen between MMP-8 and PI, GI, and GBTI ( $p < 0.05$ ;  $r = 0.31$ ,  $r = 0.38$ ,  $r = 0.31$ , respectively). In contrast, a statistically insignificant correlation was found between TNF- $\alpha$  and all clinical parameters (PI, GI, PD, GBTI, and CAL) ( $r = 0.21$ ,  $r = 0.17$ ,  $r = 0.24$ ,  $r = 0.07$  and  $r = 0.16$ , respectively) ( $p > 0.05$ ). Moreover, the correlation between MMP-8 and TNF- $\alpha$  was not significant ( $p > 0.05$ ,  $r = 0.18$ ) (Table 2).

**Table 1.** Clinical measures before and after Phase I Treatment with NSAID (group 1) and placebo (group 2)

Clinical Measure	Group 1			Group 2			* <i>p</i>
	pretreatment	post treatment	<i>p</i>	pretreatment	post treatment	<i>p</i>	
Plaque index	1.38 ± 0.47	0.56 ± 0.31	0.001	1.27 ± 0.41	0.48 ± 0.40	0.001	0.453
Gingival index	2.13 ± 0.22	1.54 ± 0.19	0.001	2.02 ± 0.09	1.49 ± 0.36	0.001	0.588
Probing depth (mm)	3.64 ± 0.50	2.92 ± 0.40	0.001	3.67 ± 0.58	2.95 ± 0.50	0.001	0.815
GBTI	3.03 ± 0.30	1.31 ± 0.49	0.001	2.82 ± 0.28	1.27 ± 0.70	0.001	0.705
CAL (mm)	3.67 ± 0.90	3.67 ± 0.89	0.317	4.08 ± 1.02	4.071 ± 0.02	0.180	0.240
TNF-α (pg)	4.23 ± 2.03	4.04 ± 1.38	0.570	4.07 ± 1.58	3.84 ± 2.29	0.754	0.785
MMP-8 (pg)	3085.64 ± 348.19	2213.43 ± 791.85	0.001	1654.61 ± 415.86	1548.31 ± 678.16	0.569	0.003

Data is presented as mean ± standard deviation, n = 16.

GBTI – gingival bleeding time index; CAL – clinical attachment loss; Comparison before and after treatment (Wilcoxon rank test)

\* *P* – Comparison of post treatment values of groups (Mann-Whitney *U* test).

**Table 2.** Correlations between MMP-8, TNF-α and the clinical parameters

Clinical parameter	MMP-8		TNF-α	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
PI	0.31	0.034*	0.21	0.168
GI	0.38	0.009*	0.17	0.261
PD (mm)	0.25	0.09	0.24	0.107
GBTI	0.31	0.037*	0.07	0.609
CAL (mm)	0.32	0.027	0.16	0.272

\* *P* < 0.05, statistically significant correlation (Pearson's correlation test).

## Discussion

Periodontal disease results from a colonization of pathogenic bacteria and activation of host-derived lytic enzymes [28]. The importance of the host response in tissue damage has been highlighted by studies demonstrating that inhibition of prostaglandins can significantly reduce the amount of periodontal destruction [29, 30]. Prostaglandins have been shown to promote inflammation and are a potent stimulus for connective tissue loss and bone resorption [31]. Based on these findings, many studies have focused on NSAIDs in the modulation of the host inflammatory response. Pelletier and Pelletier [32] examined the effects of two NSAIDs, nimesulide and naproxen, on the breakdown of the proteoglycan matrix and metalloprotease synthesis of human osteoarthritic

cartilage. These NSAIDs significantly reduced proteoglycan degradation and stromelysin synthesis *in vitro*. Salmon et al. [33] reported that leukocyte accumulation is inhibited by the NSAIDs indomethacin and flurbiprofen.

In this study, subjects with CP used tenoxicam as an adjunct to phase I periodontal treatment for 10 days. We found a significant improvement in all clinical parameters except CAL for both NSAID and placebo treatment; pre- and post-therapy values were not significantly different between the two groups, which can be attributed to the limited samples sizes. These findings suggest that tenoxicam treatment has no therapeutic effect on periodontal parameters whereas in some studies, a short time effect was found [34]. The improvements after therapy, therefore, are primarily attributable to mechanical instrumentation and oral hygiene. Moreover, the difference in baseline MMP-8 levels between the two groups could be attributed to the severity of the inflammation.

Multiple studies have examined the effect of NSAIDs on periodontal status. Vogel et al. [35] reported that sulindac does not reduce gingival inflammation, whereas Johnson et al. [36] found that naproxen reduces gingival inflammation. Heasman and Seymour [37] failed to demonstrate an effect of NSAIDs on PI, GI, PD, CAL or bone resorption; however, NSAIDs were shown to reduce GCF volume. Lee et al. [38] showed that NSAIDs administration did not reduce collagenase and gelatinase in human gingival tissues. Moreover, to them, in contrast, low dose doxycycline was effective. Additionally, a 3<sup>rd</sup> group of subjects receiving a combination of the two drugs showed a synergistic effect in reducing the matrixmetalloproteinases.



MMP-8 (collagenase-2) plays a key role in connective tissue destruction. Several studies have demonstrated higher levels of collagenase activity in the GCF of progressive periodontitis patients compared to stable and gingivitis patients [39–42]. MMP-8 at physiological (low) levels presumably provides a protective function against periodontal pathogens [43]. Kinane et al. [44] reported that MMP-8 levels in GCF decrease after phase I therapy, but the reduction reaches significant levels only after 8 weeks or longer. In our study, MMP-8 levels were reduced in both the NSAID and placebo groups at 30 days after phase I treatment, but it was significant only in the NSAID group. The latter findings suggest that one of the benefits of adjunctive tenoxicam may be to shorten the healing period by inhibiting MMP-8 activity. Moreover, in our study, no adverse effects were observed due to Tenoxicam intake. In contrast, some investigations have shown that some adverse effects such as esophagus, stomach/duodenum, small intestine and colon damage could be seen due to NSAIDs intake [45]. Barracchini et al. [46] found that meloxicam and indomethacin can inhibit the activities of MMPs from human arthritic synovial fluid *in vitro*. Williams et al. [47] suggested that flurbiprofen given in conjunction with periodontal treatment has a positive clinical and therapeutic effect. Interpretation of studies examining MMP-8, however, must be undertaken with some caution, since the results may reflect differences related to the MMP-8 antibodies used in immunoassays [48, 49].

Several studies have suggested that enhanced TNF- $\alpha$  production is an important factor in

periodontal disease progression based on its increased expression in inflamed gingiva and relatively high levels in the GCF of periodontitis patients [50, 51]. Rossomando et al. [52] reported that TNF- $\alpha$  might be a risk marker for periodontitis because of its increased levels prior to clinical attachment loss. We found no significant difference in TNF- $\alpha$  levels between our experimental groups, both before and after treatment. In the present study, TNF- $\alpha$  values observed in the control group might indicate the presence of subclinical gingival inflammation. Lee et al. [53] also reported that TNF- $\alpha$  may be a predictor of periodontitis. In addition, Erdemir et al. [54] examined the effect of non-surgical periodontal treatment on GCF TNF- $\alpha$  levels and reported a demonstrable decrease at 6 months after initial therapy. We observed a nonsignificant reduction in TNF- $\alpha$  levels after therapy and did not find an adjunctive benefit of tenoxicam. Thus, tenoxicam does not appear to provide additional inhibition of TNF- $\alpha$  early in wound healing. Similar to our results, Keçeci [55] reported that naproxen with phase I treatment does not further inhibit TNF- $\alpha$  over 6 weeks.

Within the limits of the study, the clinical benefits were demonstrated of SRP with or without an NSAID (tenoxicam) in systemically healthy patients with chronic periodontitis after the initial phase of periodontal therapy. Additional inhibition of MMP-8, but not TNF- $\alpha$ , in the GCF was observed. Further studies are needed to clarify the adjunctive effect of Tenoxicam on periodontal treatment.

**Acknowledgements.** We are thankful to Dr. Mark Reynolds from the Faculty of Dentistry, Baltimore, University of Maryland, USA, for his valuable help in editing this paper.

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Conflict of interest: None declared

Received: 22.05.2013

Revised: 19.09.2013

Accepted: 23.07.2014