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ORIGINAL ARTICLE

Effect of meloxicam on gingival crevicular fluid IL-1beta and IL1 receptor antagonist levels in subjects with chronic periodontitis, and its effects on clinical parameters

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Abstract The aim of the present study was to determine the effects of meloxicam after initial periodontal treatment on interleukin-1beta (IL-1β) and IL-1 receptor antagonist (IL-1ra) in gingival crevicular fluid (GCF) and clinical parameters in the chronic periodontitis patients. Data were obtained from 30 patients with chronic periodontitis. Fifteen chronic periodontitis patients received 7.5 mg meloxicam, and 15 patients received placebo tablets in a 1×1 regimen for 1 month. All subjects were nonsmokers and had not received any periodontal therapy. The plaque index (PI), gingival index (GI), probing depth (PD), and clinical attachment level (CAL) were recorded. The GCF was collected using a paper strip: eluted and enzyme-linked immunoabsorbent assays (ELISAs) were performed to determine the cytokine levels. The clinical data and GCF samples were obtained after periodontal therapy and 1 month after periodontal therapy. The PI, GI, PD, and GCF IL-1ra decreased significantly ($p<0.05$) in meloxicam group at first month when comparing the initial levels. While decrease of the PI was statistically significant in control group ($p<0.05$), statistically significant changes were not determined in the other clinical parameters and GCF cytokine levels ($p>0.05$). There were no significant differences between two groups in any of the investigated parameters. Our observations did not reveal any influence of meloxicam on levels of IL-1β and IL-1ra in chronic periodontitis. Additional clinical studies are advisable to determine whether COX-2 selective drugs alter periodontal disease outcome with greater safety.

Keywords Chronic periodontitis · Meloxicam · IL-1β · IL-1ra

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Introduction

Chronic periodontitis is characterized by inflammatory destruction of connective tissues, loss of periodontal attachment, and resorption of alveolar bone. The products of the inflammatory response occurring within the periodontium during disease can be found in gingival crevicular fluid (GCF). Monitoring of the presence of such components may be of value in evaluating periodontal disease status or outcomes of periodontal therapy [1]. A principal mediator of the inflammatory responses is the cytokine interleukin-1 (IL-1) [4]. IL-1 occurs in two forms as IL-1α and IL-1β, of which IL-1β appears to be the most potent agent having a catabolic effect on bone approximately tenfold compared to IL-1α. In addition to the two agonist molecules, a third member of the IL-1 group was purified and designated IL-1 receptor antagonist (IL-1ra) [10]. Its only known function is to bind to IL-1 receptors, blocking IL-1 and preventing signal transduction [20].

IL-1β is important in periodontal disease due to its potency in inhibiting bone formation and enhancing bone resorption [4, 22], and stimulating the production of prostaglandin E2, collagenase, and proteinases [4]. Previous

studies have reported that IL-1β is usually found in significant amount in GCF samples taken from patients with chronic periodontitis [1, 5]. Recently, IL-1ra has been found in gingival tissue biopsies [16] and in GCF [3, 13, 20].

There is increasing evidence that the administration of nonsteroidal antiinflammatory drug (NSAIDs), which inhibits cyclooxygenase (COX), may retard the loss of supporting alveolar bone in animal models [2, 11] and humans [14, 15]. Additionally, retrospective human studies have shown less periodontal destruction in subjects chronically ingesting various antiinflammatory drugs in comparison to controls [15]. The recent discovery that COX exists 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 [18].

The aim of this study was to investigate the effect of selective COX-2 inhibitor (meloxicam) administration on the clinical parameters of the chronic periodontitis and on the GCF IL-1β and IL-1ra levels.

Materials and methods

Study population

This study was designed in double-blind, and placebo-controlled manner. A total of 30 patients (11 men, 19 women; aged 20 to 55 years) diagnosed with chronic periodontitis with at least four sites with clinical attachment level (CAL) ≥4 mm and a probing depth (PD) ≥5 mm and one of them being in the anterior region were selected for the study. Exclusion criteria included (1) presence of diseases where administration of NSAIDs would be contra-indicated, (2) presence of systemic illness or conditions which affected 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 6 months. Informed consent was obtained from all the patients, and the Medical Ethics Committee of the Cumhuriyet University approved the study protocol (the ethical committee number: 2003/9).

Clinical study design

The patient's periodontal status was determined using the following clinical parameters: plaque index (PI) and gingival index (GI) [25], PD, and CAL. PD and CAL were measured with a Williams periodontal probe. One blinded researcher (H.T.) performed all the clinical measurements. All patients were given nonsurgical periodontal treatment and instructed on daily plaque control.

After the treatment, the patients in meloxicam group received 7.5 mg meloxicam tablets in a 1×1 regimen for a month. The placebo tablets prepared by Bilim Ilac Sanayi ve Ticaret A.S. (Istanbul, Turkey) were administered in the same way to patients in the placebo group. All patients were instructed to take the drug at the same time of the day and not to take any other drug during 30 days period without informing their periodontist and about the side effects of the drug.

The GCF sampling and clinical measurements were recorded at baseline after periodontal treatment and 1 month later. Only the upper anterior teeth were included in the study to improve the access and to reduce the risk of salivary contamination during these processes.

Crevicular fluid sampling

Each sample site was carefully isolated using cotton rolls to avoid saliva contamination because saliva contains both IL-1β and IL-1ra. Supragingival plaque and saliva were removed with cotton pellets. The paper strip (Periopaper, Pro Flow, Amityville, NY, USA) was placed in the pocket until mild resistance was felt and then left in place for 30 s. The strip was then placed in an Eppendorf tube and immediately frozen at −70°C until the day of analysis. In the case of visible contamination with blood, the strips were discarded. One GCF sample was obtained from each chronic periodontitis patient.

Analysis of cytokine production

On the day of assay, the sample strips used for the IL-1 assays were added to 200 μl of Hank's buffered salt solution containing 1% bovine serum albumin (Sigma, St Louis, MO, USA). The GCF samples were eluted from the strips by centrifugal method. The amount of IL-1β and IL-1ra in the GCF was determined by using specific enzyme-linked immunoabsorbent assays (Biosource Int., Camarillo, CA, USA) with sensitivity limits 1 and 4 pg/ml, respectively. The average recovery of IL-1β and IL-1ra spiked to different levels in samples throughout the range of the assay from various media had been determined to be 84–100 and 98–106%, respectively. The assays were carried out in accordance with the manufacturer's instructions. After the color development was stopped, the optical density was measured using a microtiter plate computerized reader set to a wavelength of 450 nm. The GCF cytokine levels were calculated from the standard curve and defined as picograms per site for total cytokine levels. The sites with cytokine levels below the limits of assays' detection ability were scored as 0 pg/site.

Analysis of data

Of the study groups, the PD, CAL, PI, and GI values and the IL-1β and IL-1ra levels measured at baseline and 1 month later were analyzed with independent and dependent t tests as appropriate, but the baseline GI value of the study groups was analyzed with Mann–Whitney test. Correlation of crevicular IL-1β and IL-1ra levels and PD, CAL, PI, and GI values were analyzed with Pearson's correlation test. Significance was determined at the $p<0.05$ level.

Results

The mean age of meloxicam group was 34.6 years (range 20–55 years, SD±10.9), whereas the mean age of placebo group was 33.8 years (range 24–51 years; SD±8.6). While the meloxicam group was comprised of six men and nine women, the placebo group was comprised of five men and ten women. Any adverse reaction associated with the medication did not occur.

Figures 1, 2, and 3 show the baseline and the 1-month PD, CAL, PI, and GI values and the IL-1β and IL-1ra levels of the meloxicam and placebo groups. There were no significant differences in both the baseline and the 1-month PD and CAL values between the meloxicam and placebo groups ($p>0.05$). There was no significant difference between the baseline and the 1-month CAL values of the meloxicam group ($p>0.05$). There were no significant differences between the baseline and the 1-month PD and CAL values of the placebo group ($p>0.05$). In the meloxicam group, the 1-month PD value was significantly lower than the baseline PD value ($p>0.05$).

There were no significant differences in both the baseline and the 1-month PI and GI values between the study groups ($p>0.05$). There was no significant difference between the baseline and the 1-month GI values of the placebo group ($p>0.05$). In the meloxicam group, the 1-month PI and GI values were significantly lower than the baseline PI and GI values ($p>0.05$). In the placebo group, the 1-month PI value was significantly lower than the baseline PI value ($p>0.05$).

While IL-1ra was detected in all GCF samples (100%), IL-1β was detected in 66% of all the samples. There were no significant differences in both the baseline and the 1-month IL-1β and IL-1ra levels between the study groups ($p>0.05$). There was no significant difference between the baseline and the 1-month IL-1β levels of the meloxicam group ($p>0.05$). In the meloxicam group, the 1-month IL-1ra levels were significantly lower than the baseline IL-1ra levels ($p<0.05$). There was no significant difference between the baseline and the 1-month IL-1β and IL-1ra levels of the placebo group ($p>0.05$).

Table 1 shows Pearson's correlation coefficients. In the meloxicam group, at both the baseline and 1-month measurements, there were moderate positive correlations between the PI value and the IL-1β level ($r=0.43$ and 0.34, respectively) but not reaching statistical significance ($p>0.05$). There was moderate positive correlation between the GI and the IL-1β level ($r=0.34$) at 1-month measurements, and moderate correlation between the CAL and the IL-1ra ($r=0.36$) at baseline measurements, but not reaching statistical significance ($p>0.05$). In the placebo group, there was moderate positive significant correlation between the baseline PI value and the IL-1β level ($r=0.60$, $p<0.05$).

Discussions

This study investigated the changes in IL-1β and IL-1ra levels found in GCF of chronic periodontitis patients undergoing meloxicam therapy. After 1-month meloxicam therapy, we did not find any significant difference in the

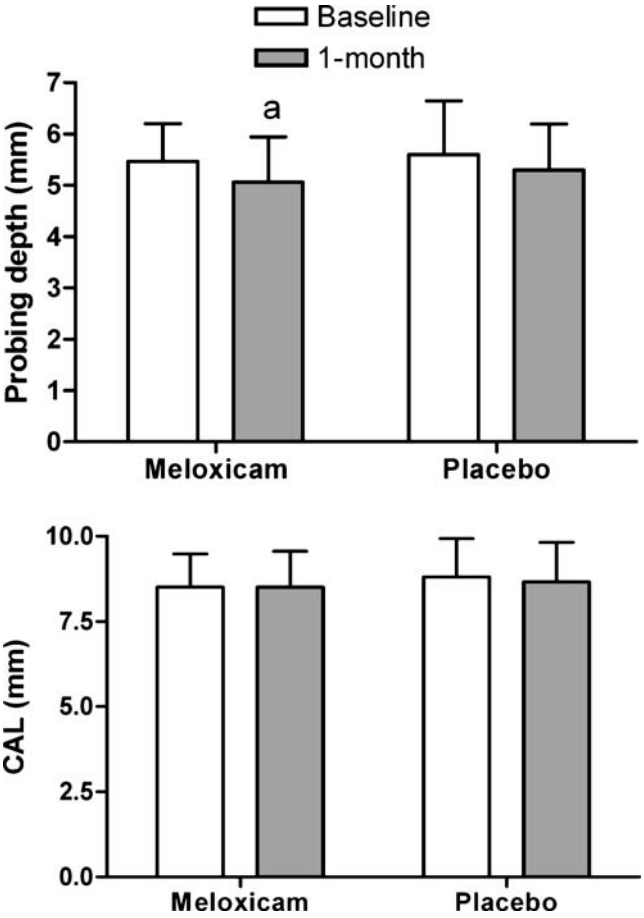

Fig. 1 PD and CAL values measured at baseline and 1 month later in meloxicam and placebo groups. Data were expressed as mean±SD. *PD* Probing depth, *CAL* clinical attachment level. [a]$P<0.05$ vs baseline value of meloxicam group

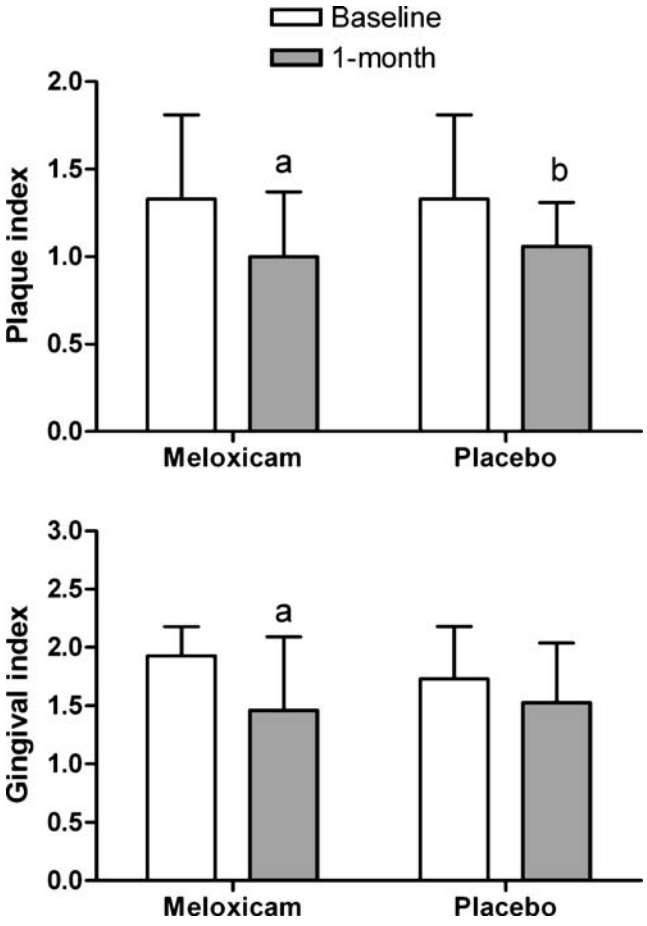

Fig. 2 Plaque and gingival index values measured at baseline and 1 month later in meloxicam and placebo groups. Data were expressed as mean±SD. [a]$P<0.05$ vs baseline value of meloxicam group. [b]$P<0.05$ vs. baseline value of meloxicam group

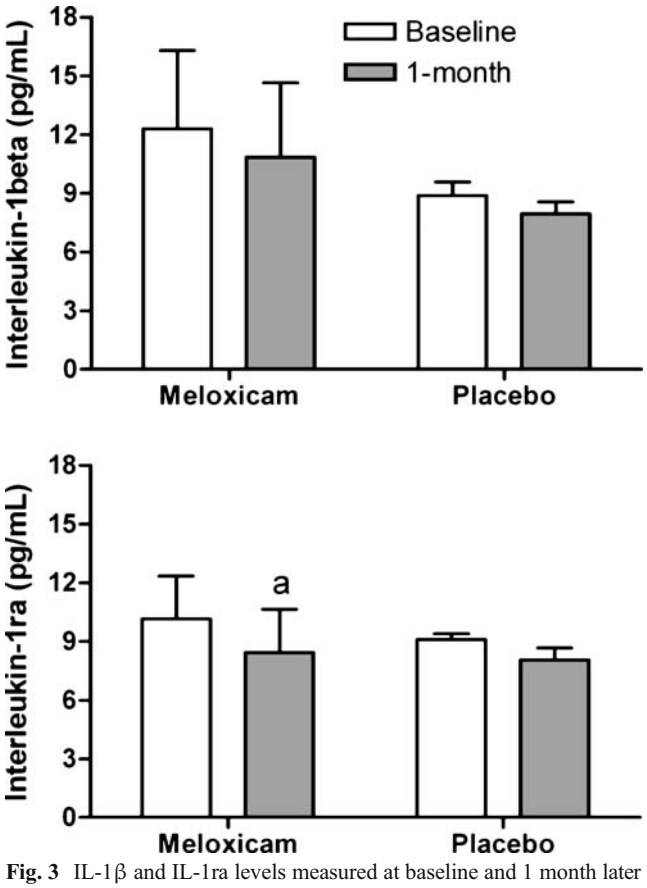

Fig. 3 IL-1β and IL-1ra levels measured at baseline and 1 month later in meloxicam and placebo groups. Data were expressed as mean±SE. [a]$P<0.05$ vs baseline value of meloxicam group

clinical parameters and the IL-1β and IL-1ra levels of the meloxicam and placebo groups.

NSAIDs are ineffective on gingival inflammation where dental plaques are present; however, after elimination of the microbial plaques, they help in resolving gingival inflammation [11]. Therefore, we preferred the use of NSAIDs after the mechanic treatment of the patients. In the present study, we found that meloxicam group decreased GI. Reddy et al. [21] also observed a significant reduction in GI for both drug and placebo groups, with no statistically significant difference between the groups. On the other hand, Williams et al. [24] reported that NSAIDs had no effect on gingival inflammation. In a recent study about the

Table 1 Associations between IL-1β and IL-1ra levels and PD, CAL, PI, and GI values measured at baseline and 1 month later in meloxicam and placebo groups

	Meloxicam group (n=15)				Placebo group (n=15)			
	Baseline		1-month		Baseline		1-month	
	IL-1β	IL-1ra	IL-1β	IL-1ra	IL-1β	IL-1ra	IL-1β	IL-1ra
PD	−0.11	−0.19	0.19	−0.08	−0.01	0.29	0.19	0.04
CAL	−0.09	0.34	0.29	0.01	−0.02	−0.09	−0.04	−0.09
PI	0.43	−0.22	0.34	−0.15	0.61[a]	−0.44	0.20	0.18
GI	0.04	0.02	0.34	0.07	−0.37	−0.02	−0.02	0.25

PD Probing depth, *CAL* clinical attachment level, *PI* plaque index, *GI* gingival index, *IL-1β* interleukin-1beta, *IL-1ra* interleukin-1 receptor antagonist
[a]$P<0.05$

use of new generation selective COX-2 inhibitors for the periodontal treatment, for 7 days subcutaneous indomethacin and COX-2 inhibitor meloxicam decreased the alveolar bone resorption and gingival inflammation similarly in the rates with the experimental periodontitis [2].

NSAID administration, by provoking COX inhibition and eicosanoid release, would be beneficial in periodontitis due to reduction of acute inflammatory changes such as local edema, cell migration, and release of free radicals [19]. In our study, even when there was a decrease in the PD value of the study groups, however, the difference between the groups was not significant. This might be the result of antiedema effect of meloxicam.

In our study, GCF was collected with filter strips along 30 s to avoid serum contamination and cytokine secretion induced by the mechanical irritation provoked by a longer collection period. Furthermore, the total amount of cytokines in GCF sample per sampling time has been suggested as a better indicator of relative GCF constituent activity rather than the GCF volume that might result in the decrease of the cytokine concentration [5, 6]. Therefore, we evaluated the total amount of the cytokines in the GCF samples.

The level of IL-1β increased in GCF in accordance with the increase of the gingival inflammation [7, 8]. Additionally, after periodontal treatment, the decrease of the inflammation together with the decrease of the IL-1β levels was shown in several studies [1, 6–8]. However, a recent study showed the slight increase in IL-1β levels in GCF after scaling and root planning after 1 month [25]. In this study, although the decrease in GCF IL-1β levels was determined in both groups, but the decrease was not found significant.

The concentration of IL-1ra in body fluids has been found to be higher than the concentration of IL-1β, and it has, therefore, been suggested that IL-1ra may be used as a marker of disease. However, the relation between clinical inflammatory response and periodontal IL-1ra concentration remains unclear [3]. Our results demonstrate that IL-1β and IL-1ra can be detected in GCF in the vast majority of patients. The initial GCF IL-1ra levels after meloxicam and placebo administrations were found 9.6 and 9.1 ng/ml, respectively. The GCF levels of IL-1ra were approximately 1,000-fold that of IL-1β. This observation was also reported by Ishihara et al. [13] and Boström et al. [3]. The decrease of the IL-1ra levels was found significant in meloxicam group. However, any significant difference was not determined between both groups for IL-1ra.

Some studies have reported positive correlations between the level of GCF inflammatory mediators and the clinical periodontal conditions [6, 13], whereas other studies have reported poor correlations between such levels and the site clinical status [5, 9]. In this study, the GCF levels of both the cytokines did not present strong correlation with clinical parameters. This could be related with the limited area of GCF sampling. To eliminate the risk of contamination with saliva, the GCF was only collected from the vestibular aspects of maxillary incisors, and this area exhibited less gingival inflammation and plaque accumulation. Probing depth showed the general cumulative effect of the periodontal disease; therefore, the PD and the cytokine levels did not show any correlation.

The present study failed to show any effect of cytokine levels of a selective COX-2 inhibitor after periodontal treatment in chronic periodontitis patients. There may be several explanations for these findings. The small number of sites examined (one site each in patients) and the time between two GCF samples (1 month) may be all probably insufficient to detect significant trends in IL-1 levels before and after meloxicam following periodontal treatment. Moreover, the side effects resulting from NSAIDs administration are a severe limitation to the routine use of these drugs in the treatment of periodontitis. Thus, further research is warranted to clarify the role of selective COX-2 inhibition as a therapeutic adjunct in the treatment of chronic inflammatory periodontitis.

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