Original Article

Effects of a Cyclic NSAID Regimen on Levels of Gingival Crevicular Fluid Prostaglandin E_2 and Interleukin-1β: A 6-month Randomized Controlled Clinical Trial

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Abstract

Background: Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for inflammation control and pain relief. However, while the adjunct use of NSAIDs is avoided for periodontal therapy because of related side effects, cyclic administration of NSAIDs may reduce or eliminate these effects. We evaluated the effect of a cyclic diclofenac potassium (DP) regimen on clinical parameters and levels of prostaglandin E_2 (PGE_2) and interleukin-1β (IL-1β) in the gingival crevicular fluid (GCF) of individuals with periodontitis. **Materials and Methods:** The study protocol was approved by the Ethics Committee (2000/071). Forty-one individuals with chronic periodontitis (33 men, 8 women) were divided into two groups (test and control) after initial periodontal therapy. During this 6-month, randomized, double-blind, placebo-controlled study, test (n = 28) and control (n = 13) groups were administered a cyclic regimen of DP (50 mg, twice daily) or placebo. Clinical measurements and GCF sample collections were made at baseline, 2, 4, and 6 months. GCF levels of PGE_2 and IL-1β were determined using enzyme immunoassay and enzyme-linked immunoassay kits, respectively. **Results:** At baseline, no significant differences existed between groups for plaque indices, gingival indices, bleeding on probing, probing depth (PD), or attachment levels ($P > 0.05$). Compared with the control group, cyclic regimen in the test group suppressed increased levels of PGE_2 found in GCF at the end of the study ($P < 0.05$). Significant differences for PD and relative attachment gain were also noted in favor of the test group ($P < 0.05$). **Conclusions:** These results suggest that a cyclic regimen of DP may be efficacious in the management of chronic periodontitis in adults.

Keywords: *Anti-inflammatory agents, cytokines, gingival crevicular fluid, nonsurgical therapy, periodontitis*

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Introduction

Bacteria and their products in dental plaque are primary etiologic factors of periodontal disease. Host response against these factors mediates periodontal tissue breakdown and severity of periodontal disease. Understanding the role of host response in periodontal disease pathogenesis has led to the concept of "host modulation therapy" being emphasized in several studies which aims to manipulate different pathways of host response and to restore equilibrium between anti- and pro-inflammatory mediators.[1-3]

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Prostaglandin E_2 (PGE_2) is an inflammatory arachidonic acid-derived mediator implicated in inflammatory processes of many chronic illnesses, including periodontal disease.[4] Elevated levels of PGE_2 have been shown in gingival crevicular fluid (GCF) and gingival tissue of patients with periodontitis.[5] PGE_2 is also involved in

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resorption of alveolar bone, which has a key role in the progression of periodontal disease.[6-8]

PGE_2 can be formed in several cell types,[9] and its expression can be induced by pro-inflammatory cytokines such as interleukin (IL)-1β.[10] A highly potent cytokine, IL-1β, is produced extensively by macrophage–monocytes in response to various stimuli, including bacterial components such as lipopolysaccharides.[11] As a local mediator of tissue destruction in periodontitis, IL-1β exerts other biologic activities including, but not limited to, inhibition of bone formation,[12] stimulation of Prostaglandin E (PGE) and thromboxane syntheses,[13] and stimulation of collagenase and protease production.[14] IL-1 is present in elevated amounts in crevicular fluid adjacent to sites of gingival inflammation and periodontal destruction.[15]

In addition to reduction of bacterial load with mechanical periodontal treatment, local and systemic administration of subantimicrobial doses of tetracycline derivatives, bone-sparing drugs (e.g., bisphosphonates), and anti-inflammatory agents (e.g., nonsteroidal anti-inflammatory drugs or NSAIDs) have been studied extensively and shown to be effective as an adjunctive host modulation therapy in the management of periodontal disease.[2,3] Several NSAID compounds, which inhibit both arachidonic acid metabolism and cyclooxygenase (COX), have been tested for treatment of periodontal disease using different modes of administration.[16-18] Results from these studies suggest that NSAIDs inhibit production of COX products in gingiva and can affect the progression of periodontal bone loss.[19] Selective NSAIDs primarily inhibit one of the main isoforms (COX-1 or COX-2) whereas nonselective NSAIDs inhibit both forms of COX. COX inhibition can cause gastrointestinal[20] and cardiovascular side effects.[21] As such risk factors should be taken into account, NSAID use and its effects should be observed closely, and periods of administration and drug dosage should be rearranged, if necessary.[22]

The use of NSAIDs as an adjunct to periodontal therapy is avoided because of side effects. Diclofenac is a nonselective NSAID[23] and may possess considerable potency against inflammation and gives rise to metabolites with anti-inflammatory actions.[24] Adverse events associated with diclofenac use are, in general, mild and transient. We hypothesize that NSAIDs may have beneficial effects on the periodontium if preventive strategies (e.g., changes to duration of administration) are incorporated to eliminate their side effects. Therefore, we aimed to evaluate the effects of cyclic diclofenac potassium (DP) regimen on clinical parameters and levels of PGE_2 and IL-1β in GCF.

Materials and Methods

Ethical considerations

The study protocol was approved by the Ethics Committee of the University (Project Number 2000/071). The study cohort comprised patients attending the periodontology department. Written informed consent was obtained from all individuals before participation in the study.

Study protocol and selection of patients

Individuals were selected from a group of patients with chronic periodontitis and had received initial periodontal therapy, including scaling and root planning (SRP), as well as oral hygiene instruction at least 6 weeks before study commencement. The inclusion criteria for all patients in the maintenance were (i) no history of asthma, cardiovascular, renal and gastrointestinal or other chronic diseases, hypersensitivity to diclofenac or other NSAIDs; (ii) had not received antibiotics within the last 6 months before study commencement; (iii) had not received NSAIDs within 1 month or phenytoin or calcium antagonists within 3 months; (iv) no history of pregnancy, lactation, or inadequate birth control; and (v) had more than 16 teeth, at least four of which had a probing depth (PD) ≥5 mm, and radiographic evidence of alveolar bone loss of 30%–50%.[25]

This study utilized a randomized, controlled, double-blind, parallel-group design to investigate the 6-month effect of a cyclic regimen of DP on clinical parameters of periodontal disease and levels of PGE_2 and IL-1β in GCF [Figure 1]. Forty-nine patients were divided into two groups: 28 patients in the test group (ten smoker, eleven nonsmoker, and seven ex-smoker individuals) and 13 patients in the control group (six smoker, five nonsmoker, and two ex-smoker individuals). Randomization of individuals with periodontitis was ensured using a coin toss.

Test and control groups were administered either DP (50 mg) or placebo gel caps, respectively, b. i. d. for 6 months in a cyclic regimen. Administration of DP or placebo was undertaken from baseline to 2 months, no drug (DP or placebo) was administered from 2 to 4 months, and then, DP or placebo therapy was reinstituted from 4 to 6 months. To promote compliance, each patient was recalled monthly and instructed on oral hygiene. SRP was not carried out during these recalls. During the screening period (at baseline, 2, 4, and 6 months), all individuals underwent physical examinations and biochemical analyses (blood chemistry, complete blood count, urinalyses, and pregnancy testing).

After isolation of teeth with a cotton roll, plaque index (PI)[26] was recorded and supragingival

plaque was removed. The teeth were air-dried gently, and subsequently, the first sample of GCF volume (GCFV-1) was collected for PGE_2 analyses. Ten minutes later, a second sample (GCFV-2) was collected for IL-1β analyses. All samples were collected with a standard paper strip (Periopaper® GCF Strips, Pro Flow Incorporated, North Haven, CT) inserted into the sulcus until mild resistance was felt for 30 s. Amount of sample collected was quantified with Periotron 8000 (Harco Electronics Incorporated, Mississauga, ON, Canada). The strips from the four sampling sites of each patient were placed in 250 μL of a PGE_2 enzyme immunoassay buffer solution (Cayman Chemical Company, Ann Arbor, MI), and for the second sampling procedure, four strips from the same patient sampling sites were placed in 500 μL of phosphate-buffered saline (pH 7.6) and stored at −30°C until assay. At the time of analyses, tubes were vortex mixed to maintain elution of GCF from strips at room temperature.

Customized acrylic stents were fabricated and used to ensure probing from the same sites. Clinical examinations were carried out by one operator. After sampling, PD and relative attachment level (RAL) were measured using the Williams periodontal probe (Hu-Friedy, Chicago, IL), and bleeding on PI scores[27] of the sampling area was recorded. GCF sampling and clinical evaluation were conducted at each screening period.

A commercial competitive enzyme immunoassay and an enzyme-linked immunoassay (ELISA) were carried out according to manufacturers' recommendations to quantify PGE_2 and IL-1β levels, respectively (Cayman Chemical Company, Ann Arbor, MI, USA). Results were read using a microplate reader at a wavelength of 405 nm.

Statistical analyses

Values for individual selected sites were averaged for each individual at each time point. For normalization of biochemical values, natural logarithmic transformations were undertaken. Mann–Whitney U-test was used to evaluate intergroup differences. At baseline, clinical parameters, PGE_2 level, IL-1β level, age, and smoking status were accepted covariates; univariate analyses were used to evaluate intergroup differences. The nonparametric Friedman test was used to determine the intragroup significance of changes for clinical parameters, concentrations, and total amounts of PGE_2 and IL-1β between time periods. If intergroup differences were significant ($P < 0.05$), multiple comparisons were analyzed using the Bonferroni-adjusted Wilcoxon signed-rank test. Values of $P < 0.05$ were considered significant.

Results

Eight patients (six in the test group and two in the control group) dropped out of study voluntarily. The study was completed in 33 men and eight women aged 32–60 (44.4 ± 7.4) years. None of the patients experienced a side or adverse effect related to use of DP.

The intergroup differences were insignificant at baseline ($P > 0.05$). The intragroup significances of changes between time periods are presented in Table 1.

Clinical periodontal parameters of patients are presented in Table 2. Compared with baseline, PD was reduced by approximately 31%, 42%, and 51% in the test group ($P < 0.05$) and 24%, 36%, and 39% in control group ($P < 0.05$) at 2, 4, and 6 months, respectively. During the screening period, significant differences in PD and RAL were found between test and control groups, except at baseline ($P < 0.05$). PI scores were high for both groups, and inter- and intra-group differences were not significant ($P > 0.05$). Between baseline and 6-month examinations, BOP index scores showed a significant reduction for both groups ($P < 0.05$).

A significant reduction was not observed for GCFV-1, or concentration or total amount of PGE_2 between baseline and 6-month measurements in test and control groups ($P > 0.05$). The intergroup differences of concentration and total amount of PGE_2 were significant ($P < 0.05$) at the end of the study [Table 3]. A significant reduction of GCFV-2 was observed between baseline and 6 months in the test group ($P < 0.05$) [Table 4]. Significant intergroup differences of GCFV-2 were found at 2 and 6 months ($P < 0.05$). Statistically outlying data were

Table 1: Evaluation of intragroup changes in clinical periodontal parameters, gingival crevicular fluid levels of prostaglandin E_2 and interleukin-1 beta (concentrations and total amounts) and gingival crevicular fluid volume using the Friedman test

	Test (*n*=28)	Control (*n*=13)
PD	0.000**	0.000**
RAL	0.000**	0.000**
PI	0.744	0.264
BPI	0.000**	0.016*
GCFV-1	0.654	0.951
GCFV-2	0.022*	0.399
PGE_2 (pg/μL)	0.782	0.198
PGE (pg)	0.181	0.485
IL-1β (pg/μL)	0.000**	0.145
IL-1β (pg)	0.033*	0.145

**P<0.001, *P<0.05. PD=Probing depth; RAL=Relative attachment level; PI=Plaque index; BPI=Bleeding on probing index; GCFV=Gingival crevicular fluid volume; IL=Interleukin; PGE_2=Prostaglandin E_2

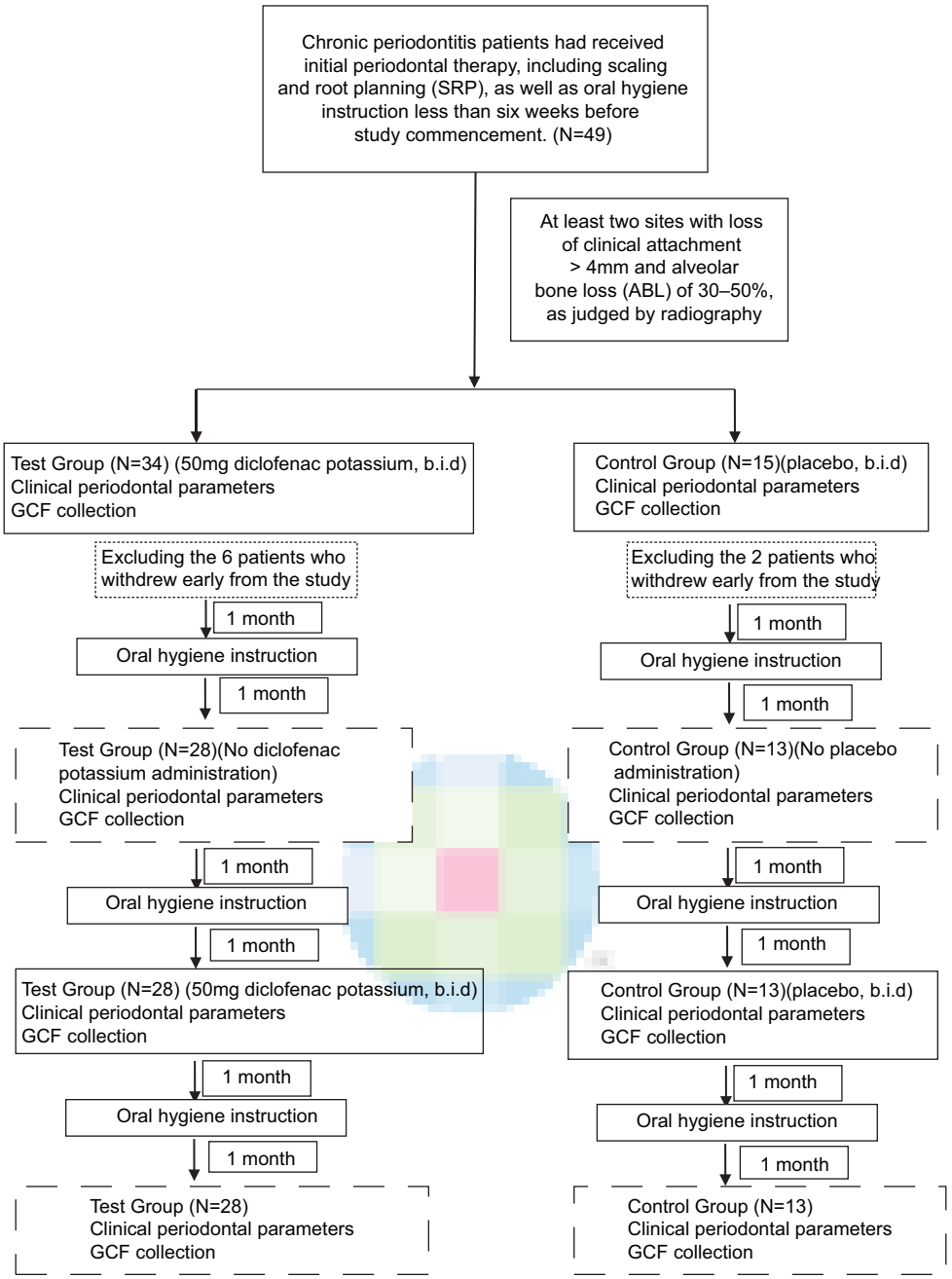

Figure 1: Study flowchart

Table 2: Descriptive data of clinical periodontal parameters in test and control groups

	Test (*n*=28)		Control (*n*=13)	
	Mean±SD	**Median (range)**	**Mean±SD**	**Median (range)**
PD				
Baseline	5.26[a]±0.41	5.25 (4.75-6.25)	5.36[e]±0.50	5.25 (4.75-6.75)
2-month*	3.58[b]±0.65	3.50 (2.50-5.00)	4.03[f]±0.65	3.25 (2.00-5.00)
4-month	3.05[c]±0.67	2.87 (2.00-4.75)	3.38[g]±0.83	3.25 (2.00-5.00)
6-month*	2.57[d]±0.52	2.50 (1.75-3.75)	3.25[h]±0.78	3.00 (2.00-5.00)
RAL				
Baseline	8.33[j]±1.03	8.12 (7.00-12.00)	8.25[p]±1.10	8.00 (6.25-9.75)
2-month*	6.83[k]±1.10	6.87 (5.00-10.25)	7.30[r]±1.22	6.75 (5.00-8.75)

Contd...

Table 2: Contd...

	Test (*n*=28)		Control (*n*=13)	
	Mean±SD	Median (range)	Mean±SD	Median (range)
4-month*	6.34[m]±1.08	6.25 (4.75-8.50)	6.86[s]±1.22	6.50 (5.00-8.75)
6-month*	5.98[n]±0.83	6.00 (4.50-8.50)	6.71[t]±1.29	6.50 (5.00-8.75)
PI				
Baseline	2.11±0.92	2.25 (1.00-3.00)	2.01±0.73	2.00 (1.00-3.00)
2-month	1.96±0.74	2.00 (1.00-3.00)	2.23±0.88	2.50 (0.00-3.00)
4-month	2.12±0.71	2.00 (1.00-3.00)	2.09±0.79	2.25 (1.00-3.00)
6-month	1.97±0.70	2.00 (1.00-3.00)	1.80±0.77	1.50 (1.00-3.00)
BPI				
Baseline	1.76[u]±0.62	2.00 (0.25-3.00)	1.57±0.67	2.00 (0.00-2.00)
2-month	1.18[v]±0.71	1.12 (0.00-2.00)	1.07±0.89	1.00 (0.00-3.00)
4-month	0.67[w]±0.71	0.50 (0.00-2.00)	1.00±0.75	1.00 (0.00-3.00)
6-month	0.55[z]±0.66	0.37 (0.00-2.00)	0.84±0.86	0.75 (0.00-3.00)

Univariate analysis: Baseline data were accepted covariate and comparison of changes in clinical parameters between test and control groups at 2, 4, and 6 months, **P*<0.05; Bonferroni adjusted Wilcoxon signed-rank test: Comparison of baseline and 2, 4, and 6 months in both groups (a:b, a:c, a:d, b:c, b:d, e:f, e:g, e:h, f:g, j:k, j:m, j:n, j:k, j:m, k:n, p:r, p:s, p:t, r:s, r:t, u:v, u:w, w:ş, v:w, v:z), *P*<0.05. PD=Probing depth; RAL=Relative attachment level; PI=Plaque index; BPI=Bleeding on probing index; SD=Standard deviation

Table 3: Descriptive data of gingival crevicular fluid volume, as well as concentration and total amount of prostaglandin E2 in test and control groups

	Test (*n*=28)		Control (*n*=13)	
	Mean±SD	Median (range)	Mean±SD	Median (range)
GCFV-1 (µL)				
Baseline	1.54±0.92	1.26 (0.46-3.57)	1.71±0.62	1.84 (0.50-2.60)
2-month	1.52±0.66	1.61 (0.51-2.76)	1.71±0.86	1.60 (0.51-3.18)
4-month	1.54±0.88	1.20 (0.52-3.50)	1.69±0.61	1.78 (0.22-2.73)
6-month	1.42±0.82	1.23 (0.25-3.75)	1.64±0.73	1.85 (0.40-2.92)
lnPGE2 (pg/µL)				
Baseline	0.56±0.28	0.50 (0.07-1.56)	0.48±0.22	0.39 (0.25-0.97)
2-month	0.47±0.23	0.44 (0.03-1.12)	0.58±0.19	0.56 (0.17-0.93)
4-month	0.59±0.27	0.61 (0.08-1.34)	0.64±0.37	0.59 (-0.06-1.42)
6-month*	0.50±0.24	0.50 (0.03-1.07)	0.65±0.19	0.68 (0.33-0.90)
$lnPGE^2$ (pg)				
Baseline	0.67±0.33	0.55 (0.24-1.46)	0.67±0.24	0.69 (0.23-1.16)
2-month	0.61±0.29	0.57 (0.09-1.40)	0.76±0.27	0.76 (0.28-1.12)
4-month	0.71±0.32	0.66 (0.12-1.55)	0.82±0.39	0.85 (0.26-1.62)
6-month*	0.58±0.26	0.59 (0.10-1.15)	0.82±0.29	0.77 (0.33-1.27)

Univariate analysis: Baseline data were accepted covariate and comparison of changes in clinical parameters between test and control groups at 2, 4, and 6 months, **P*<0.05. GCFV=Gingival crevicular fluid volume; PGE_2=Prostaglandin E_2; ln=Natural logarithm; SD=Standard deviation

Table 4: Descriptive data of gingival crevicular fluid volume, as well as concentration and total amount of interleukin-1 beta in test and control groups

	Test (*n*=26)		Control (*n*=13)	
	Mean±SD	Median (range)	Mean±SD	Median (range)
GCFV-2 (µl)				
Baseline	1.92[a]±0.89	1.90 (0.73-3.90)	1.78±0.73	1.85 (0.40-2.92)
2-month*	1.73±0.72	1.74 (0.41-3.07)	2.17±0.99	1.93 (0.59-3.81)
4-month	1.65±0.89	1.57 (0.26-3.61)	1.97±0.90	1.98 (0.19-3.45)
6-month*	1.48[a]±0.71	1.37 (0.34-2.73)	1.93±0.85	2.10 (0.50-3.29)
lnIL-1β (pg/µL)				
Baseline	1.41[b,c]±0.38	1.42 (0.63-2.10)	1.39±0.53	1.52 (0.54-2.09)
2-month	1.73[b]±0.37	1.67 (0.99-2.46)	1.78±0.44	1.70 (1.19-2.61)

Contd...

Table 4: Contd...

	Test (*n*=26)		Control (*n*=13)	
	Mean±SD	Median (range)	Mean±SD	Median (range)
4-month	1.69±0.40	1.59 (1.02-2.74)	1.68±0.39	1.73 (0.98-2.39)
6-month	1.79[c]±0.31	1.82 (0.97-2.47)	1.89±0.48	1.99 (1.32-2.84)
lnIL-1β (pg)				
Baseline	1.65[d]±0.39	1.73 (0.78-2.35)	1.59[e]±0.59	1.59 (0.78-2.52)
2-month	1.91±0.41	1.94 (1.28-2.85)	2.07±0.55	1.92 (1.28-3.19)
4-month	1.83±0.42	1.76 (0.95-2.80)	1.90±0.46	1.80 (1.31-2.82)
6-month	1.90[d]±0.29	1.89 (1.38-2.51)	2.12[e]±0.43	2.10 (1.50-2.86)

Univariate analysis: Baseline data were accepted covariate and comparison of changes in clinical parameters between test and control groups at 2, 4, and 6 months, **P*<0.05. Bonferroni adjusted Wilcoxon signed-rank test: Comparison of baseline and 2, 4, 6 months in both groups (a:a, b:b, c:c, d:d, e:e), *P*<0.05. GCFV=Gingival crevicular fluid volume; IL=Interleukin; ln=Natural logarithm; SD=Standard deviation

determined in two GCF samples that were collected for IL-1β evaluation in the test group which were discarded from analyses. Intragroup levels of IL-1β concentration and the total amount increased significantly from baseline to 6 months ($P < 0.05$). The cyclic regimen of DP was found to suppress an increase in concentration and total amount of IL-1β by the end of the study ($P < 0.05$).

Discussion

NSAIDs exert their anti-inflammatory effects by inhibiting the COX pathway and PGE_2 synthesis. Elevated levels of PGE_2 have been shown at periodontitis sites[28,29] and decrease with resolution of inflammation,[16,30] thereby emphasizing the potent mediator role of PGE_2 in the pathogenesis of periodontal disease.[9] Effects of NSAIDs as an adjunct to SRP on PGE levels and periodontal parameters have been examined in both human and animal studies.[18,19,31-33] However, this is the first study investigating effects of a cyclic DP regimen on levels of PGE_2 and IL-1β in GCF of patients with chronic periodontitis in the recall period.

In an animal study administering NSAIDs for 6 months, Offenbacher *et al*.[19] showed PGE_2 inhibition in GCF by the end of the 1st month. Vardar *et al*.[31] demonstrated short-term use of a nonselective NSAID as an adjunct significantly decreased PGE_2 levels in GCF. In the present study, PGE_2 levels were also decreased in the test group, but in control group, PGE_2 levels increased throughout the study. Possibly, this can be attributed to our study population, all of whom were treated with SRP at least 6 weeks before study baseline. Increased PGE_2 levels in the placebo group were also shown in a study by Jeffcoat *et al*.[18] comparing NSAID use at 3 and 6 months and in another short-term study by Vardar *et al*.[31] evaluating the effects of NSAIDs. They suggested that the outcome of periodontal treatment should be evaluated by biochemical markers and periodontal parameters.[31] Offenbacher *et al*.[19] suggested high levels of PGE_2 may be an indicator of loss of attachment. Elevated levels may also be the result of continuing bacterial stimulation and associated subclinical processes.

Lipopolysaccharide derived from periodontopathogens may stimulate IL-1β production in the periodontium, which is responsible for most bone resorption. Elevated levels of IL-1 β have been observed at sites of gingival inflammation and periodontal destruction[15] and correlate with disease severity. Resolution of periodontal inflammation is generally accompanied by decreased levels of IL-1β.[34] In our study, clinical outcomes improved in terms of decreased PD and RAL. In contrast to some previous studies,[34,35] IL-1β levels increased in both groups throughout the study. This feature may be related to our study design, as we did not perform SRP during the 6-month study period. IL-1β levels were slightly lower in the test group compared with placebo group, suggesting DP may have an inhibitory effect on this GCF biomarker.

In conflict with our results, some studies have reported that IL-1β levels do not change significantly after periodontal therapy. Teles *et al*.[36] investigated relationships between GCF biomarkers, periodontal parameters, and subgingival microbiota in patients with chronic periodontitis. In shallow sites (considered to be clinically healthy in patients with periodontitis), significantly higher levels of IL-1β were observed compared with healthy controls. Inflammatory mechanisms undetectable by clinical means could be the reason for elevated levels of IL-1β.[36] In a gingivitis study based on short-term accumulation of plaque, IL-1β levels were shown to be elevated before clinical signs of inflammation were observed.[37] Microbiological evaluation revealed higher proportions of periodontopathogens in periodontitis patients, whose clinically healthy sites were also suggested to be colonized with higher levels of these species.[36] Bacterial stimuli from subgingival biofilms of patients with periodontitis have been hypothesized to influence subclinical inflammation and expression of GCF

biomarkers, and these sites could carry a higher risk for initiation and progression of periodontal disease.[36] Although improvements in PD and RAL were observed after periodontal therapy and requirements of oral hygiene were emphasized during each examination period in our study, reductions in PI scores were not clinically significant. Elevated levels of IL-1β observed may also be the result of existing bacterial stimuli and subclinical processes that should be investigated in future studies.

Increased expression of IL-β with different severities of periodontal disease and elevated levels of IL-1β in shallow sites of patients with severe periodontitis have been reported.[38] In addition, genetic-based characteristics of the host have been suggested to play a role in IL-1β expression.[39] Gilowski *et al*.[40] reported that the total amount of IL-1β and IL-1 receptor antagonist (IL-1ra) was higher in patients with periodontitis. The ratio of IL-1β/IL-1ra was also high in patients with periodontitis, such that IL-1ra expression was too low to reciprocate IL-1β release.[40] In our study, the genetic characteristics modifying expression of IL-1 and IL-1ra[40] may have affected IL-1β levels, as well as the initiation and progression of disease, regardless of the therapy applied.

The complex network of cytokines, signal transduction pathways, and interactions among cells have important roles in inflammation and all may regulate expression of PGE_2-synthesizing enzymes.[9] Numerous cell types contribute to PGE_2 synthesis in gingival connective tissue, but levels of PGE_2 release differ among cell types.[9,41,42] An *in vitro* study focusing on the expression of PGE syntheses in samples from individuals with periodontitis used tumor necrosis factor (TNF)-α and IL-1β as inflammatory stimuli in cell cultures.[9] IL-1β stimulation increased levels of PGE synthase in cultures of gingival fibroblasts and smooth muscle cells. In cultures of endothelial cells and mast cells, which revealed basal expression of PGE synthase and COX-2, PGE synthesis was unaffected by IL-1 β stimuli.[9] IL-1 β was localized in CD45+ cells in gingival biopsy specimens, and by producing cytokines such as IL-1 β,[43] CD45+ cells have been suggested to regulate PGE_2 production in sites of periodontal disease.[9] Bacterial stimulation, as reflected by PI (reduced in a nonsignificant manner throughout the study period), may have increased levels of CD45+ cells that regulate PGE_2 production, resulting in elevated IL-1 β levels observed in our study. Elevated IL-1 β levels and slightly decreased levels of PGE may suggest that DP possesses limited effects on control of PGE-synthesizing cell types and pathways.

Studies evaluating the effects of DP on GCF biomarkers PGE_2 and IL-1β are lacking, so comparison of results could not be made. Topical[44]and systemic[45] administration of diclofenac has failed to affect serum levels of IL-1 β in animal studies. Dugina *et al*.[46] reported that diclofenac use for 6 months did not change serum levels of cytokines (IL-1, IL-6, TNF-α) in a study comparing antibodies to TNF-α in patients with rheumatoid arthritis. In the present study, the nonselective NSAID diclofenac was hypothesized to suppress IL-1 β levels; a GCF biomarker believed to reflect local inflammation. Our results showed increased levels of IL-1 β throughout the study could be attributed to the pharmacologic mechanism of action of diclofenac, which is incompletely understood. Diclofenac may operate in different anti-inflammatory pathways in which IL-β levels may not be directly affected.

Adverse effects of long-term use of NSAIDs have been established.[20,21] In the present study, administration of a cyclic NSAID regimen at 2-month interval over a 6-month study period did not cause any adverse effects as reported by patients or detected by laboratory evaluations. Diclofenac use after periodontal treatment is preferred because dental biofilms have been shown to reduce the effects of NSAIDs.[8] All clinical parameters, except PI, decreased in both groups throughout our study. Meticulous oral hygiene procedures were stressed during all examination periods, but patients' ability to remove plaque efficiently was not improved. Resolution of the clinical signs of inflammation (less bleeding on brushing and color changes of gingiva) might have led patients to think that the periodontium had "healed," resulting inadequate control of plaque and reflected in a nonsignificantly changed PI. Reductions in PD and RAL in the test group were significantly different from controls, suggesting an effect of DP.

Clinical results of our study appear to be promising, but NSAIDs have rebound effects[16] after cessation of use that may restrict their effects on disease progression. Rebound effects on clinical parameters were not prominent, but biologic markers seemed to be affected. Rebound effects of NSAID use were observed in PGE_2 levels at 4 months, consistent with other studies reporting that application time restricts improvements elicited by NSAIDs.[16]

Although our study included a relatively small sample size and short observation period, this randomized, double-blind, and controlled study is the first to investigate a cyclic regimen of DP after nonsurgical periodontal therapy in recall period. No side or adverse effects of orally administrated NSAIDs were observed or reported by patients who participated in the study.

Long-term studies with larger patient groups may clarify possible effects of cyclic DP regimen administration as an adjunct to periodontal therapy or in recall period.

Conclusions

The present study suggests a cyclic regimen of DP administration restricts increases in IL-1 β levels in the GCF and with respect to PD and RAL improves clinical outcomes in patients with chronic periodontitis. Reduction of PGE_2 levels in GCF was not significant and exact mechanisms of action for DP could not be clarified. Further studies employing different doses and administration regimens of DP are necessary to understand its exact mechanism of action on periodontal tissues.

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Conflicts of interest

There are no conflicts of interest.

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