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The gingival crevicular fluid ciprofloxacin level in subjects with gingivitis and periodontitis, and its effects on clinical parameters

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Objectives: The purpose of this study, conducted on patients with gingivitis and periodontitis, was twofold: to find out the serum and gingival crevicular fluid concentration of ciprofloxacin, which is a common drug used effectively against *Actinobacillus actinomycetemcomitans* and to determine the effects of ciprofloxacin administration on clinical parameters.

Method: A total of 32 adult patients, consisting of 16 subjects with gingivitis and 16 subjects with untreated chronic periodontitis, were included in the study. The subjects were divided into four groups: group I included eight subjects with chronic gingivitis who had not previously received any ciprofloxacin; group II included eight subjects with chronic gingivitis to whom three doses of ciprofloxacin were administered (Siprosan 500 mg) to establish adequate gingival crevicular fluid and serum concentrations of the agent; group III consisted of eight subjects with chronic periodontitis who had not received any ciprofloxacin; group IV included eight subjects with chronic periodontitis to whom three doses of ciprofloxacin were administered to establish adequate gingival crevicular fluid and serum concentrations of the agent. All patients were systemically healthy, free of pain and reported no current medication usage. Each patient was treated with scaling and/or root planing using specific hand instruments under local anesthesia. Gingival index, plaque index and clinical attachment levels of the teeth were used to determine the clinical condition of the subjects and findings were recorded at the beginning, seventh day, 21st day and third month of the study. Serum ciprofloxacin level was measured in venous blood. Approximately 5 ml of venous blood was drawn from subjects in groups II and IV using a standard venipuncture technique. Gingival crevicular fluid samples were sampled from six interproximal sites with six paper strips in the posterior region of upper jaw (excluding third molar) and all gingival crevicular fluid and serum samples were evaluated by high-performance liquid chromatography.

Results: The serum concentrations of ciprofloxacin at the first and 72nd hour were not significantly different in subjects with periodontitis compared to subjects with gingivitis. But the gingival crevicular fluid concentrations of ciprofloxacin at the same hours were significantly high in subjects with periodontitis compared to subjects with gingivitis. Both subjects with gingivitis and periodontitis had signi-

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ficantly higher ciprofloxacin levels in the gingival crevicular fluid than in serum. The application of ciprofloxacin did not have any positive or statistically significant effect upon the clinical parameters of the subjects with gingivitis. On the other hand, a significant decrease in the clinical attachment level scores of the subjects with periodontitis (group IV) was observed compared to group III in the 21st day and third month.

Conclusion: According to these results, the use of ciprofloxacin as an alternative drug in subjects with periodontitis but not gingivitis can be recommended. However, long-term studies are also needed to assess the effects of ciprofloxacin on clinical parameters.

Gingivitis and periodontitis are primarily bacterial infections caused by diverse group of micro-organisms (1–3). The prevalence and severity of these diseases can be reduced by mechanical plaque removal or a variety of systemic and topically applied antimicrobial agents aimed at selectively removing or inhibiting pathogenic bacteria (4, 5).

Mechanical removal, which is either non-surgical or surgical, is time-consuming, operator and patient-dependent and difficult to master. The use of chemotherapeutic agents as adjuncts to mechanical and surgical debridement is compelling (6, 7).

Destructive periodontal disease is a concern because of the potential damage to the dentition and the financial burden of treatment. It is generally agreed that micro-organisms residing in the periodontal pockets are responsible for periodontitis, but uncertainty exists regarding the exact mechanisms by which periodontal tissues are destroyed (8). Approximately 500 bacterial taxa inhabit periodontal pockets, which provide a moist, warm, nutritious and anaerobic environment for microbial colonization and multiplication. The abundance and diversity of periodontal pocket micro-organisms depend upon several factors, including effectiveness of oral hygiene procedures, pocket depth, degree of gingivitis, flow of gingival crevice liquid, type of interacting microbes and viruses, transmission rate of microbes from other individuals and the antimicrobial efficacy of the host immune response (9).

During the past two decades, dentists and microbiologists have embraced periodontal antibiotic therapy, as evidence for bacterial specificity in periodontitis has accumulated and strengthened (10). It was indicated that systemic antimicrobial agents could serve as useful adjuncts for eradicating invasive periodontal pathogens (11). For this reason, the tetracycline family of antibiotics, which reaches higher levels in gingival crevicular fluid than in serum, is widely used to inhibit the periodontal pathogens (12, 13). In recent years, aside from tetracyclines, it has been shown that ciprofloxacine is distributed at significantly higher levels in gingival crevicular fluid than in serum (14, 15). It was also found that ciprofloxacine is highly active against periodontal pathogens such as *Actinobacillus actinomycetemcomitans*, enteric rods and pseudomonads (9, 16). Although some studies have been undertaken on the gingival crevicular fluid ciprofloxacine level (14, 15), we could not find any published article about the effects of ciprofloxacin administration on clinical parameters. So the purpose of this study, conducted on patients with gingivitis and periodontitis, was twofold: to find out the serum and gingival crevicular fluid concentration of ciprofloxacin, which is a common drug used effectively against *A. actinomycetemcomitans*, and to determine the effects of ciprofloxacin administration on clinical parameters.

Materials and methods

Selection of patients

A total of 32 adult patients, 22 males and 10 females with a mean age of 39.2 years who applied to the Department of Periodontology (Ataturk University, Faculty of Dentistry, Turkey) for the periodontal treatment were admitted. Half of the subjects had gingivitis without any radiographic evidence of bone loss but had varying degrees of gingival inflammation (17). The remaining 16 subjects had untreated chronic periodontitis (18), characterized with radiographic evidence of bone loss and clinical attachment loss in excess of 5 mm in more than four premolar and/or molar sites. All subjects were non-smokers, generally healthy and were not on any antibiotic or other drug treatment for at least 6 months prior to the study. The subjects who were pregnant and received periodontal therapy were excluded. A detailed description of the study was given to each subject.

Clinical examination and measurement

To determine the clinical situation of the subjects, gingival index (19), plaque index (20) and clinical attachment levels of the teeth were measured and recorded at the beginning of the study. Plaque index and gingival index were recorded at four sites per tooth (buccal, mesial, distal and lingual) and clinical attachment level was assessed by distance from base of pocket to the cemento-enamel junction in six sites (mesiobuccal, distobuccal, midbuccal, mesiolingual, midlingual and distolingual) on each tooth by using a manual probe (Hu-Friedy Manufacturing Inc., Chicago, IL, USA). The subjects were divided into four groups: group I included eight subjects with chronic

gingivitis who had not previously received any ciprofloxacin; group II included eight subjects with chronic gingivitis, to whom three doses of ciprofloxacin were administered (Siprosan 500 mg, Drogsan, Ankara, Turkey) to establish adequate gingival crevicular fluid and serum concentrations of the agent; group III consisted of eight subjects with chronic periodontitis who had not received any ciprofloxacin; group IV included eight subjects with chronic periodontitis to whom three doses of ciprofloxacin were administered to establish adequate gingival crevicular fluid and serum concentrations of the agent. Following the initial examination and measurement, all patients were motivated and instructed to use manual toothbrush and dental floss but not to use mouth rinses or irrigating solutions for the duration of the study (21). Full-mouth scaling and/or root planing using newly sharpened hand instruments (scalers and Gracey curettes) were performed under local anesthesia by a periodontist (AT) after the gingival crevicular fluid and serum samples were collected at the 72nd hour.

The subjects in groups II and IV continued to administer ciprofloxacin for 7 days. The administration of ciprofloxacin was limited to a 7-day duration to minimize the potential unfavourable side-effects. Gingival crevicular fluid and serum samples were collected at the 1st, 72nd and 168th hour after the first dose of ciprofloxacin, and all clinical parameters were repeated in the seventh day, 21st day and third month by an experienced periodontist (RO) who was blind to the subjects' scaling and/or root planing and the group selection.

Collection and measurement of gingival crevicular fluid and serum samples

Gingival crevicular fluid and serum samples were collected according to the method of Conway *et al.* (15). After selection of the sites (interproximal crevicular sites from selected teeth in the maxillar posterior region excluding third molar), the sites were isolated with cotton rolls and the gingival tissues were air dried to avoid contamination with saliva. A positioning paper strip (Periopaper, ProFlow, Inc., Amityville, NY, USA) was inserted into the crevice until mild resistance was felt, and left in place for 1 min. A total of six paper strips were obtained from each subject. These paper strip samples were then pooled with 75-μl aliquots of 100 μM glycine, pH 3, using the method of Offenbacher (22). The strips were discarded in the case of visible contamination with blood. The gingival fluid volume was measured with a Periotron 8000 (Harco Periotron 8000, ProFlow, Inc.) that was calibrated with an established method by Preshaw *et al.* (23). Gingival crevicular fluid ciprofloxacin levels were calculated by dividing the content of each sample pool by total volume. Serum ciprofloxacin level was measured in venous blood. Approximately 5 ml of venous blood was drawn from subjects in groups II and IV using a standard venipuncture technique. All gingival crevicular fluid and serum samples were prepared according to the method of Jim *et al.* (24), and stored at −40°C until the analysis. The ciprofloxacin concentrations in gingival crevicular fluid and serum were measured by high-performance liquid chromatography, using a method described by Jim *et al.* (24). The samples were evaluated from a 10 microns U-Bondapack C-18 cartridge at ambient temperature with a mobile phase consisting of acetonitrile : 0.1 M sodium dihydrogen phosphate (20:80%, v/v) adjusted to pH 3.9 with phosphoric acid, and at a flow rate of 2.5 ml/min. The samples were monitored on a fluorescence detector using an excitation and emission wavelength of 280 and 455 nm, respectively.

Statistical analysis

Statistical significance of data for all clinical parameters within a group was determined with the paired *t*-test. Significant differences between the two groups were determined with the Student's *t*-test. Changes were considered significant at the $p < 0.05$ levels.

Results

As shown in Table 1, the subjects with periodontitis (groups III and IV) had high levels of gingival index, plaque index, and clinical attachment level scores compared to subjects with gingivitis (groups I and II). The groups with gingivitis (groups I and II) had classical symptoms of the infection, such as edema, erythema, bleeding and exhibited a clinical attachment levels of 2.2 ± 0.30 and 2.3 ± 0.56 mm, respectively.

The groups with periodontitis demonstrated significantly increased gingival crevicular fluid volume (5.3 ± 0.54 μl) compared to subjects with gingivitis (3.1 ± 0.32 μl). The serum and gingival crevicular fluid concentrations of ciprofloxacin in groups II and IV are presented in Tables 2 and 3. The serum concentration of ciprofloxacin was not significantly different in subjects with periodontitis compared to subjects with gingivitis in the 1st, 72nd and 168th hour. But, the gingival crevicular fluid concentration of ciprofloxacin was significantly higher in subjects with periodontitis as compared to subjects with gingivitis in

Table 1. Clinical parameters for each group at initial

	GI	PI	CAL
Group I	1.8 ± 0.25	2.0 ± 0.46	2.2 ± 0.30
Group II	1.9 ± 0.42*	2.2 ± 0.12*	2.3 ± 0.56*
Group III	2.4 ± 0.21¶	2.7 ± 0.23¶	6.8 ± 0.34¶
Group IV	2.7 ± 0.26*,¶	2.9 ± 0.52*,¶	7.2 ± 0.61*,¶

Values are expressed as a group mean ± SD.
*No significant difference between groups I and II and between groups III and IV ($p > 0.05$).
¶Significant difference between groups I and II and groups III and IV, respectively ($p < 0.05$).
GI, gingival index, PI, plaque index; CAL, clinical attachment level.

Table 2. Ciprofloxacin concentration in gingival crevicular fluid and serum at 1st and 72nd hour

	Gingival crevicular fluid		Serum	
Group	1st hour (μg/ml)	72nd hour (μg/ml)	1st hour (μg/ml)	72nd hour (μg/ml)
Group II	2.2 ± 0.21	2.9 ± 0.56	0.3 ± 0.24	0.8 ± 0.32†
Group IV	2.6 ± 0.69¶	3.9 ± 0.45¶	0.4 ± 0.36*	0.9 ± 0.25*,†

Values are expressed as a group mean ± SD.
*No significant difference between groups II and IV ($p > 0.05$).
¶Significant difference between group II and IV ($p < 0.05$)
†Significant difference between gingival crevicular fluid and serum ($p < 0.05$).

Table 3. Ciprofloxacin concentration in gingival crevicular fluid and serum at 72nd and 168th hours after scaling and/or root planing

	Gingival crevicular fluid		Serum	
Groups	72nd hour (μg/ml)	168th hour (μg/ml)	72nd hour (μg/ml)	168th hour (μg/ml)
Group II	2.9 ± 0.56	2.3 ± 0.54†	0.8 ± 0.32	0.7 ± 0.56
Group IV	3.9 ± 0.45¶	3.4 ± 0.36¶,†	0.9 ± 0.25*	0.8 ± 0.11*

Values are expressed as a group mean ± SD.
*No significant difference between group II and IV ($p > 0.05$).
¶Significant difference between group II and IV ($p < 0.05$).
†Significant difference between 72nd and 168th hours ($p < 0.05$).

the 1st, 72nd and 168th hour. In both groups, gingival crevicular fluid ciprofloxacin levels were significantly higher than serum levels ($p < 0.05$). Prior to scaling and root planing, the mean gingival crevicular fluid ciprofloxacin concentrations at the 1st and 72nd hour in groups II and IV were 2.2 ± 0.21, 2.6 ± 0.69 and 2.9 ± 0.56, 3.9 ± 0.45 (μg/ml), respectively. After the scaling and/or root planing, the mean gingival crevicular fluid ciprofloxacin concentration at the 168th hour in groups II (2.3 ± 0.54 μg/ml) and IV (3.4 ± 0.36 μg/ml) was lower than at the 72nd hour (group II, 2.9 ± 0.56 μg/ml; group IV, 3.9 ± 0.45 μg/ml) (Table 3) ($p < 0.05$). However, the serum ciprofloxacin concentration remained unchanged after the performance of scaling and/or root planing. In order to determine the effects of ciprofloxacin upon clinical parameters, the plaque index, gingival index and clinical attachment level scores of all four group of subjects at the initial, 7th day, 21st day and third month were measured. The plaque index, gingival index and clinical attachment levels scores of subjects with periodontitis (groups III and IV) were significantly higher than subjects with gingivitis (groups I and II) initially (Table 1, $p < 0.05$). After the scaling and/or root planing and the administration of ciprofloxacin, the plaque index and gingival index scores of each group in the 7th day, 21st day and third month were significantly different when they were compared to the initial measurements ($p < 0.05$). But, when the group I, which included the subjects who did not receive ciprofloxacin, and group II, which consisted of the subjects to whom ciprofloxacin were administered, were compared, no significant differences were found between these groups in the 7th day, 21st day and third month ($p > 0.05$). As shown in Table 4, significant statistical differences were observed between clinical attachment level scores of groups III and IV in the 21st day and third month.

When the data was expressed as clinical parameters (plaque index and gingival index), ciprofloxacin did not reveal any positive or significant effect for the subjects with gingivitis. On the other hand, a significant decrease in the clinical attachment levels scores of subjects with periodontitis (group IV) was observed.

Discussion

Conventional periodontal therapy, with its focus on mechanical debridement of bacterial plaque, usually prevents the progression of periodontal attachment loss. In some individuals, however, periodontal breakdown continues despite careful attention to debridement (25). Some periodontal pathogens such as *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Dialister pneumosintes*, *Tannerella forsythensis* and *Treponema denticola* can escape from debridement by invading the soft tissue wall of the periodontal pocket (9, 26, 27). Other gram-negative anaerobic rods, some gram-positive bacteria and even enteric rods/pseudomonas may also play a role in the etio-pathogenesis of periodontitis (9). So patients with periodontitis may benefit from the usage of systemic antibiotics, topical antibiotics and topical antiseptics. Systemic antibiotics may be required for eradication and prevention of the periodontal infections by *A. actinomycetemcomitans* and other pathogens that invade subepithelial periodontal tissue or colonize extra-dental domains from which they may translocate to periodontal sites (10). For this reason, ciprofloxacin, a bactericidal agent that inhibits bacterial DNA gyrase, was widely used against *A. actinomycetemcomitans* recently. This study determined that gingival crevicular fluid and serum level of ciprofloxacin were high in those subjects with gingivitis and periodontitis (groups II and IV). The mean gingival crevicular fluid level of this agent was higher than its serum level at the 1st, 72nd and 168th hour. When the gingival crevicular fluid ciprofloxacin level of subjects with periodontitis was compared to those of with gingivitis, the former was found to be significantly high. But, after the scaling and/or root planing, it was observed that this level decreased at the 168th hour in both groups (group II and IV). Aithal *et al*. (14) showed that significant gingival crevicular fluid and serum

Table 4. Clinical parameters for each group after scaling and/or root planing and administration of ciprofloxacin

	Initial	7th day	21st day	3rd month
GI				
Group I	1.8 ± 0.25	0.5 ± 0.20	0.6 ± 0.23	0.9 ± 0.12¶
Group II	1.9 ± 0.42	0.5 ± 0.21*	0.7 ± 0.42*	1.0 ± 0.31*·¶
Group III	2.4 ± 0.21	0.6 ± 0.12*	0.7 ± 0.42*	0.8 ± 0.49¶
Group IV	2.7 ± 0.26	0.7 ± 0.34**	0.5 ± 0.54**	0.9 ± 0.51**·¶
PI				
Group I	2.0 ± 0.46	0.8 ± 0.33	0.9 ± 0.36	1.0 ± 0.28¶
Group II	2.2 ± 0.12	0.7 ± 0.26*	0.8 ± 0.54*	1.1 ± 0.45*·¶
Group III	2.7 ± 0.23	0.9 ± 0.28	0.9 ± 0.21	1.2 ± 0.13¶
Group IV	2.9 ± 0.52	0.9 ± 0.58**	0.9 ± 0.47**	1.1 ± 0.35**·¶
CAL				
Group I	2.2 ± 0.30		2.1 ± 0.54	2.1 ± 0.47
Group II	2.3 ± 0.56		2.0 ± 0.34*	2.0 ± 0.22*
Group III	6.8 ± 0.34		5.9 ± 0.56	5.9 ± 0.18¶
Group IV	7.2 ± 0.61		5.3 ± 0.54†	5.3 ± 0.26†·¶

Values are expressed as a group mean ± SD ($n = 8$ each group).
*No significant difference between group I and II ($p > 0.05$).
**No significant difference between group III and IV ($p > 0.05$).
¶Significant difference between initial and 7th, 21st days and 3rd month ($p < 0.05$).
†Significant difference between group III and IV ($p < 0.05$).
GI, gingival index, PI, plaque index; CAL, clinical attachment level.

ciprofloxacin levels were observed in healthy subjects and in subjects with inflamed periodontal disease, and Conway *et al*. (15) have recently demonstrated that the mean gingival crevicular fluid levels of this agent were four to five times higher than its serum level. This was confirmed in the present investigation, too. However, our mean gingival crevicular fluid ciprofloxacin concentration in subjects with periodontitis was higher than those reported by Conway *et al*. (15). Although the precise reason for such discrepancy is unknown, one could think that the sampling method or the properties of selected subjects could be responsible; our samples were taken at 1st, 72nd and 168th hours. Concerning the sampling method, Eley and Cox (28) also argued that residual fluid in the periodontal pocket could be diluted with newly formed inflammatory exudates during longer gingival crevicular fluid collection. In our study, our sampling took 1 min and was performed as a single periopaper strip. Our study suggested that the concentration of ciprofloxacin in subjects with periodontitis is greater than in subjects with gingivitis at 72nd hours. After the scaling and/or root planing, a statistically significant decrease in gingival crevicular fluid ciprofloxacin levels of groups II and IV at 168th hours was observed. We tend to think that inflammation could contribute to enhancing the delivery of ciprofloxacin to the periodontal pocket, as subjects with periodontitis had higher gingival index scores than those with gingivitis. Seymour *et al*. (29) suggested that lymphocytes and polymorphonuclear leukocytes (PMNs) are the predominantly infiltrating leukocytes at the early lesion of gingivitis, and inflamed periodontium is densely infiltrated by PMNs as well. Easmon and Crane (30) reported that the ciprofloxacin levels inside PMNs are usually four to eight times higher than the levels in the extracellular medium. It is our view that ciprofloxacin level might attain higher concentrations in the gingival crevicular fluid of untreated subjects than treated ones.

The present study demonstrated that non-surgical therapy had a significant effect on the plaque index, gingival index and clinical attachment levels scores. The effect of scaling and/or root planing on clinical parameters is generally related to the initial scores (31). After the scaling and/or root planing and administration of ciprofloxacin, significant statistical differences in each groups' plaque index and gingival index scores were observed in the 7th day, 21st days and third month when compared with the initial measurements ($p < 0.05$). However, when the plaque index and gingival index scores of groups I and II were compared between groups in the 7th day, 21st days and third month, statistical differences were not observed between the two groups ($p > 0.05$) and a similar finding was also observed between groups III and IV ($p > 0.05$). But, in the 21st day and third month, statistical differences were observed between the clinical attachment level scores of groups III and IV.

It is reported that most of the periodontal diseases are plaque-associated disorders that start as an overt inflammation of gingiva. If left untreated, in certain susceptible individuals, inflammation may spread to involve a deeper portion of the periodontium (32, 33). In the periodontitis, the inflammatory cell infiltrate extends laterally and further apically into the connective tissue when compared with gingivitis (34). In the present study, the reason why a discrepancy between the groups plaque index and gingival index scores in the 7th day, 21st day and third month were not observed may be attributed to the mechanical elimination of bacterial deposits from supra and subgingival tooth surfaces. The discrepancy observed in the attachment gain changes in group IV at the 21st day and third month may be due to the usage of ciprofloxacin together with the scaling and/or root planing. It can be suggested that the significant and positive correlation between clinical attachment levels and the administration of ciprofloxacin together with scaling and/or root planing is related to the ciprofloxacin retained bactericidal activity inside PMNs, which ultimately contributes to enhanced intracellular killing of susceptible bacteria.

In conclusion, considering the results of our study, the ciprofloxacin was determined at an effective concentration in gingival crevicular fluid and serum. The concentration of ciprofloxacin was significantly higher in gingival crevicular fluid than serum at all measurements. When 7th day, 21st day and third month scores were

compared, there was no statistically significant change in the plaque index and gingival index scores between each group, but clinical attachment level scores of group IV significantly reduced when compared with those of group III at the 21st day and third month. With these results the use of ciprofloxacin as an alternative drug in subjects with periodontitis (not gingivitis) can be recommended. However, long-term studies are also needed to make a more comprehensive assessment of the effects of ciprofloxacin on clinical parameters.

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