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Gingival Fluid Ciprofloxacin Levels at Healthy and Inflamed Human Periodontal Sites

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Abstract

Background—PMNs take up and accumulate ciprofloxacin. This may allow them to enhance the delivery of this agent to the inflamed periodontium.

Methods—Cross-sectional and longitudinal approaches were used to test the hypothesis. In the cross-sectional study, seven periodontally healthy adults and eight adults with untreated periodontitis were administered three doses of ciprofloxacin (500 mg BID). Gingival fluid (GF) and serum samples were obtained after 28 hours and analyzed by HPLC. In the longitudinal study, eight adult periodontitis subjects were administered 500 mg ciprofloxacin BID for 8 days. After 28 hr, GF from four sites with 5 to 8 mm probing depths was sampled in each subject, serum samples were obtained, and two of the four sites were root planed. GF and serum were sampled again 7 days later (196 hr after the initial dose).

Results—The mean ciprofloxacin levels in the GF and serum of periodontally healthy subjects were 2.52 ± 0.22 μg/ml and 0.47 ± 0.05 μg/ml, respectively. In subjects with periodontitis, these levels were 2.69 ± 0.44 μg/ml and 0.61 ± 0.13 μg/ml, respectively. GF ciprofloxacin levels were significantly higher than corresponding serum levels in healthy and diseased subjects ($P < 0.01$), but there were no significant differences in GF or serum levels between the two subject groups. Since GF flow was significantly higher at diseased sites, however, more ciprofloxacin was distributed to these sites than to healthy sites. In the longitudinal study, GF flow at 196 hr was 16% lower at root planed sites than at untreated control sites ($P = 0.412$). The minor decrease in this index of inflammation was accompanied by a small (9%), but statistically significant ($P = 0.007$) decrease in GF ciprofloxacin levels.

Conclusion—GF ciprofloxacin levels decreased slightly at inflamed periodontal sites after root planing, but were significantly higher than serum levels even at healthy periodontal sites. Inflammation may enhance the distribution of ciprofloxacin to diseased sites, but it is not a major determinant of GF ciprofloxacin levels.

Keywords

antimicrobial delivery; fluoroquinolones; infection; inflammation; periodontitis

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INTRODUCTION

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. These infections are often related to persistent infection by invasive subgingival bacteria (1). *Actinobacillus actinomycetemcomitans*, a periodontal pathogen that possesses several virulence factors and is strongly associated with early onset periodontitis, can resist debridement and evade the host response by invading the soft tissue wall of the pocket (2,3). Systemic antimicrobial agents can serve as useful adjuncts for eradicating invasive periodontal pathogens. The tetracycline family of antibiotics, which reaches higher levels in gingival fluid (GF) than in serum (4,5), is widely used to inhibit *A. actinomycetemcomitans* and many other subgingival bacteria (6). *A. actinomycetemcomitans* is also highly susceptible to ciprofloxacin (7,8), a bactericidal agent that inhibits bacterial DNA gyrase. Like other fluoroquinolones, ciprofloxacin terminates chromosomal replication and interferes with cell division and gene expression (9).

Fluoroquinolones are taken up and accumulated by energy-dependent transporters in the plasma membrane of polymorphonuclear leukocytes (PMNs) (10). The transport process is so efficient that fluoroquinolone levels inside PMNs are usually 4- to 8-fold higher than the levels in the extracellular medium (11). Ciprofloxacin retains its bactericidal activity inside PMNs, and contributes to enhanced intracellular killing of susceptible bacteria (11,12). By acting as reservoirs for ciprofloxacin, PMNs migrating from the bloodstream could potentially enhance the delivery of fluoroquinolones to inflammatory sites. Because the inflamed periodontium is densely infiltrated by PMNs, we hypothesized that systemic ciprofloxacin might attain higher concentrations in GF from inflamed periodontal sites than it does in blood serum or GF from healthy sites. We also anticipated that scaling and root planing would decrease GF ciprofloxacin levels at inflamed sites. The results of this study partially support these hypotheses by demonstrating that ciprofloxacin attains a higher concentration in GF than in serum. However, the data suggest that inflammation is not a major determinant of ciprofloxacin levels in the periodontium.

MATERIALS AND METHODS

Study Design

Two groups of subjects were recruited: a healthy group consisting of 7 subjects with good oral hygiene and healthy gingival tissues, and a periodontally diseased group consisting of 8 subjects with untreated adult periodontitis (characterized by probe depths ≥ 5 mm and moderate to advanced bone loss in at least two quadrants). Pregnant females and patients taking anti-inflammatory agents or antibiotics were excluded from participation in the study. Subjects from both groups were administered three doses of ciprofloxacin (500 mg BID) to establish steady state tissue levels of the agent. GF and serum samples were obtained 28 hr after the first dose of ciprofloxacin to facilitate a cross sectional comparison of ciprofloxacin levels at healthy and inflamed periodontal sites. The subjects in the diseased group continued taking ciprofloxacin (500 mg BID) for another 7days, and a longitudinal, split-mouth design was employed to assess the effect of scaling and root planing on GF ciprofloxacin levels at these sites. Although this design did not permit complete resolution of inflammation, the study was limited to an 8 day duration to minimize the potential for unfavorable responses to ciprofloxacin (including induction of resistance).

Assessment of GF and serum ciprofloxacin levels in healthy and periodontally diseased subjects

GF and serum samples were collected 28 hr after the first dose of ciprofloxacin. GF from the healthy subjects was sampled from 12 interproximal sites with 12 paper strips. The sites were isolated with cotton rolls and the gingival tissues were air dried to avoid contamination with saliva. GF samples were obtained by positioning a paper strip (Periopaper®; Proflow Incorporated, Amityville, New York) at the orifice of the sulcus for 30 seconds. The paper strips were manipulated gently to avoid contamination with blood. Gingival fluid volume was measured with a Harco Periotron 6000 that had been calibrated by an established method (13). The 12 strips were pooled for elution of ciprofloxacin. In the diseased subjects, the two quadrants with the most severe periodontal destruction were selected for study. Two different sites in each quadrant were selected for GF sampling. Three paper strip samples were obtained at each site, for a total of 12 strips per subject. Paper strip samples from each quadrant were pooled, placed in microcentrifuge tubes, and stored on ice. Approximately 5 ml of venous blood was drawn from subjects in both groups, using standard venipuncture techniques. All GF and serum samples were processed with minimal delay.

Comparison of GF ciprofloxacin levels at control and root-planed sites in periodontally diseased subjects

After the 28 hr sample collection from the periodontally diseased subjects, one quadrant was randomly selected for treatment with scaling and root planing. No treatment was rendered in the other quadrant. The subjects continued taking 500 mg ciprofloxacin BID for seven additional days, returning 196 hr after the first dose of ciprofloxacin for collection of GF and serum samples.

Processing of GF and serum samples

Immediately after sample collection, ciprofloxacin was eluted from the GF sample strips. The twelve paper strip samples obtained from each healthy subjects were pooled and eluted with two 75 μl aliquots of 100 mM glycine, pH 3, using a method previously described (14). With periodontally diseased subjects, paper strip samples from control and root planed sites were pooled within each quadrant, and eluted in the same manner. Ciprofloxacin recovery from the paper strips was essentially quantitative. All GF and serum samples were prepared according to the method of Jim et al (15) and stored at −20° C until analysis.

Analysis of ciprofloxacin in GF and serum samples

The ciprofloxacin content of GF and serum were measured by HPLC, using a method described by Jim et al (15). The samples were separated by isocratic reversed phase chromatography on a C_{18} column (Waters Novapak, 5 × 100 mm). The mobile phase consisted of acetonitrile:0.1 M sodium dihydrogen phosphate (20:80% v/v) adjusted to pH 3.9 with phosphoric acid. Sample elution was monitored with a fluorescence detector, using an excitation wavelength of 280 nm and a 418 nm long pass emission filter.

GF ciprofloxacin levels were calculated by dividing the ciprofloxacin content of each sample pool by its total volume. In the cross-sectional study, the GF sample was pooled from 12 paper strip samples per subject, and the sample volume was the sum of the volumes collected with these strips. In the longitudinal study, GF samples from root-planed and untreated control sites in each subject were pooled from 6 paper strip samples each.

Statistical analysis

The independent t-test was used for between-group comparisons of serum ciprofloxacin concentration, GF ciprofloxacin concentration and GF volume in healthy and diseased subjects.

In cases of unequal variances (as indicated by the folded F-test), the Satterthwaite approximation was used. The dependent t-test was used to compare ciprofloxacin levels in serum and GF. All type I error values were Bonferroni-adjusted using the step-down method of Holm.

In the longitudinal portion of this study, a repeated-measures ANCOVA was used to analyze GF ciprofloxacin concentrations at 196 hr. The 28 hr GF ciprofloxacin concentration was the covariate in this analysis. A similar approach was used to assess between-treatment comparisons for GF volume at 196 hr, using the 28 hr value as the covariate.

RESULTS

Characteristics of the subject groups

The sites in the healthy subject group exhibited a mean probing depth (± SEM) of 2.6 ± 0.20 mm and were free of edema, erythema, bleeding, and other signs of gingivitis (Table 1). None of these subjects exhibited attachment loss. Sites in the periodontally diseased group entered the study with a mean probing depth of 5.7 ± 0.25 mm, moderate to advanced (15 to 50%) interproximal bone loss, and generalized marginal erythema and edema. The mean pooled GF volume in the healthy group (pooled from twelve 30 second paper strip samples) was 1.29 ± 0.09 μl. This was significantly lower than the diseased group (P = 0.0045), which had a mean pooled GF volume of 4.43 ± 0.53 μl.

Serum and GF ciprofloxacin levels in healthy and periodontally diseased subjects

In both the healthy and the diseased subjects, GF ciprofloxacin levels were significantly higher than serum concentrations (P < 0.01, Table 2). After tissue levels of ciprofloxacin reached steady-state (28 hr after initial dose), the mean GF ciprofloxacin concentration in the healthy group was 2.52 ± 0.22 μg/ml, while the mean serum ciprofloxacin concentration was 0.47 ± 0.05 μg/ml. The corresponding ciprofloxacin concentrations in the diseased group were 2.69 ± 0.44 μg/ml in GF and 0.61 ± 0.13 μg/ml in serum. There was no significant difference in the GF ciprofloxacin concentrations between healthy and diseased subjects (P = 1.00).

Effect of root planing on GF ciprofloxacin levels in periodontally diseased subjects

Prior to root planing (28 hr after the initial dose), the mean GF ciprofloxacin concentrations at control sites and sites destined for treatment by root planing were 2.48 ± 0.36 μg/ml, and 2.90 ± 0.54 μg/ml, respectively (Table 3). After seven additional days of ciprofloxacin therapy (196 hr after the initial dose), the mean adjusted GF ciprofloxacin concentration at the control sites (3.06 ± 0.04 μg/ml) was approximately 9% higher than at treated sites (2.81 ± 0.04 μg/ml). This difference was statistically significant (P = 0.007). Between 28 and 196 hr, the pooled GF volume at control and root planed sites decreased only slightly. At 196 hr, the mean pooled GF volume at root planed sites was 16% lower than at untreated control sites (P = 0.412, Table 4).

DISCUSSION

The effectiveness of conventional periodontal therapy is compromised by an inability to debride bacteria from infected sites. The furcation region can act as a reservoir for periodontal pathogens because it is relatively inaccessible for scaling and root planing (16–18). The dentinal tubules of teeth affected by periodontal disease can also harbor pathogenic bacteria (19). In addition, some periodontal pathogens (e.g., *A. actinomycetemcomitans*) can escape debridement by invading the soft tissue wall of the periodontal pocket (2,20). Systemic antimicrobial agents can help eradicate these inaccessible bacteria (6). Ciprofloxacin is particularly effective against many invasive pathogens because of its ability to penetrate cells

and produce bactericidal effects (21). Since GF is a transudate of serum, we expected our healthy subject group to have similar levels of ciprofloxacin in their GF and serum. However, we hypothesized that migrating PMNs could enhance the distribution of ciprofloxacin to inflamed periodontitis sites and thereby enhance the local concentration of this antimicrobial agent. The objective of this study was to test this hypothesis.

The results of our cross-sectional study suggest that inflammation has no significant impact on GF ciprofloxacin levels. The mean GF levels of this agent were 4- to 5-fold higher than its serum levels, regardless of whether the subjects' periodontal tissues were healthy or inflamed. This finding is clinically relevant, since ciprofloxacin's antimicrobial activity is dose-dependent (22). Its increased availability in GF should enhance its bactericidal effects against susceptible subgingival microorganisms. The mean GF ciprofloxacin levels observed in our subjects (2.5 to 2.7 μg/ml) were well in excess of ciprofloxacin's MIC for *A. actinomycetemcomitans* (0.010 μg/ml) (7). Despite the similarity in GF ciprofloxacin levels, the total amount of ciprofloxacin delivered to inflamed sites was greater than at healthy sites because GF flow (as reflected by GF sample volume) was significantly higher at inflamed sites. This suggests that ciprofloxacin could have been preferentially distributed to inflamed sites, but was diluted by the increased flow of GF.

In the longitudinal component of our study, root planing induced a small, but statistically significant decrease in GF ciprofloxacin levels within one week. During the course of the study, the mean pooled GF volume at root planed sites decreased by 16% relative to that of untreated control sites. However, the mean pooled GF volumes obtained from treated and control sites were not significantly different at the conclusion of the study ($p = 0.412$). Had the duration of this study been longer than 8 days, more complete resolution of inflammation may have been possible at the root-planed sites. Longer-term ciprofloxacin therapy would have increased the risk of undesirable side effects in our subjects, however.

Our cross-sectional study suggests that the total amount of ciprofloxacin perfusing inflamed periodontitis sites is greater than at healthy sites. While the cross-sectional and longitudinal studies support slightly different conclusions regarding the relationship between inflammation and local ciprofloxacin levels, it should be noted that the longitudinal study's repeated measures statistical analysis is more sensitive to small differences in GF ciprofloxacin concentrations. The statistically significant decrease in GF ciprofloxacin levels observed at root-planed sites suggests that inflammation could contribute to enhanced delivery of ciprofloxacin to the periodontal pocket. However, the small magnitude of this decrease indicates that inflammation is not a major determinant of ciprofloxacin levels in GF. Since periodontally healthy subjects also exhibited relatively high GF ciprofloxacin levels, other mechanisms appear to be involved. Gingival connective tissue and crevicular epithelium are interposed between the gingival vascular bed and the gingival crevice, so fibroblasts or epithelial cells could potentially play a role in enhancing GF ciprofloxacin levels.

Most types of subgingival bacteria found in subjects with adult periodontitis can be eliminated by mechanical debridement, so antimicrobial chemotherapy is indicated in only a relatively small proportion of periodontal patients. However, systemic antimicrobial agents can help eliminate invasive pathogens associated with early onset periodontitis or refractory periodontitis. Ciprofloxacin is highly active against *A. actinomycetemcomitans*, and is also effective against enteric rods and pseudomonads associated with advanced adult periodontitis (23,24). The agent has also been used in combination with metronidazole to treat mixed infections of anaerobic bacteria, *A. actinomycetemcomitans*, enteric rods, and pseudomonads (7,23). Aside from tetracyclines and ciprofloxacin, no other antimicrobial agents are known to distribute at significantly higher levels in GF than in serum. However, ciprofloxacin shares its basic structure with new-generation fluoroquinolones (e.g., levofloxacin and trovafloxacin)

which may be transported and distributed in a similar manner. Since these agents are much more active against gram-negative anaerobes than ciprofloxacin (25), studies of their effectiveness in the treatment of early onset or refractory forms of periodontitis are warranted.

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Table 1

Characteristics of the subject groups and their GCF collection sites

Group	Age	Bone Loss at Sites	Probing Depth	Pooled GCF Volume
Healthy (n = 7)	29.3 ± 0.97 yrs	None detected	2.6 ± 0.20 mm	1.29 ± 0.09 μL
Diseased (n = 8)	44.7 ± 3.34 yrs	Moderate-to-severe	5.7 ± 0.25 mm	4.43 ± 0.53 μL*

Results are presented as mean ± SEM.

* Diseased group pooled GCF volume was significantly different from the healthy group (P = 0.0045).

Table 2

Ciprofloxacin concentrations in GCF and serum

Group	Mean GCF Concentration	Mean Serum Concentration
Healthy	2.52 ± 0.22 μg/mL	0.47 ± 0.05 μg/mL
Diseased	2.69 ± 0.44 μg/mL	0.61 ± 0.13 μg/mL

Results are presented as mean ± SEM. In both groups, there was a significant difference between GCF and serum levels ($P < 0.01$).

Table 3

GCF ciprofloxacin concentrations at control and root-planed sites

Site category	Pretreatment level (28 hr)	Post-treatment level (196 hr*)
Untreated control	2.48 ± 0.36 μg/mL	3.06 ± 0.04 μg/mL
Root-planed	2.90 ± 0.54 μg/mL	2.81 ± 0.04 μg/mL

Results are presented as mean ± SEM.

* Post-treatment levels were adjusted for pretreatment levels, using ANCOVA. There was a significant difference between the ciprofloxacin levels at control and root-planed sites at 196 hr (P = 0.007).

Table 4

Effect of root planing on pooled GCF sample volume

Site category	Pretreatment volume (28 hr)	Post-treatment volume (196 hr*)
Untreated control	2.38 ± 0.36 μL	1.78 ± 0.22 μL
Root-planed	2.05 ± 0.19 μL	1.49 ± 0.22 μL

Results are presented as mean ± SEM.

* Post-treatment levels were adjusted for pretreatment levels, using ANCOVA. The difference between the mean post-treatment volumes was not significant (P = 0.412).