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Distribution of Systemic Ciprofloxacin and Doxycycline to Gingiva and Gingival Crevicular Fluid

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Abstract

Background—Systemic fluoroquinolones and tetracyclines reach steady-state levels in gingival crevicular fluid (GCF) that are several-fold higher than their levels in serum. The mechanism by which this occurs is unclear, but gingival fibroblasts are known to accumulate these agents. Uptake by fibroblasts could enhance their distribution to gingiva. To test this hypothesis, steady-state levels of these agents were assayed in serum, gingival connective tissue (GCT), and GCF.

Methods—Healthy subjects who needed resective periodontal surgery participated in the study. Approximately 78 hours prior to the surgical appointment, each subject began a 3-day regimen of ciprofloxacin or doxycycline. At the surgical appointment (scheduled approximately 6 hours after the last dose), samples of blood and GCT were collected. GCF samples were collected on paper strips and measured with an electronic device. Samples were extracted and analyzed by high performance liquid chromatography.

Results—Mean ciprofloxacin levels in serum, GCT, and GCF were 0.40 µg/ml, 1.38 µg/g, and 1.66 µg/ml, respectively ($P < 0.001$, $N = 9$). For doxycycline, these levels were 1.11 µg/ml, 2.03 µg/g, and 2.41 µg/ml, respectively ($P = 0.002$, $N = 8$). For both agents, the GCT and GCF levels were significantly higher than serum levels ($P < 0.05$), but not significantly different from each other.

Conclusions—Our findings suggest that fibroblasts could play an important role in the distribution of fluoroquinolones and tetracyclines to the gingiva. By accumulating these agents in GCT, fibroblasts could contribute to the relatively high levels they attain in GCF.

Keywords

Connective tissue; gingival/analysis; fibroblasts; fluoroquinolones; gingival crevicular fluid/analysis; serum/analysis; tetracyclines

Although root planing and surgical intervention are the foundation of periodontal therapy, adjunctive antimicrobial chemotherapy can improve the effectiveness of treatment in individuals with aggressive periodontitis. Pathogens associated with these disorders (e.g., *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*) are capable of invading the soft tissue wall of the pocket.^{1–3} Systemic antimicrobial therapy offers several advantages in the treatment of these infections. It can act against pathogens that have invaded soft tissue and inhibit microorganisms at other sites that are relatively inaccessible (e.g., furcations). Systemic therapy is capable of eliminating pathogens not only from periodontal sites, but also from most other sites in the oral cavity.⁴ This ability to eradicate periodontal

pathogens from the oral cavity can provide considerable therapeutic benefits, since reinfection must occur from external sources.

Among the antibiotics used in periodontics, fluoroquinolones are highly efficient in killing *A. actinomycetemcomitans*, while tetracyclines are capable of inhibiting both *A. actinomycetemcomitans* and *P. gingivalis*.^{4–6} Previous studies have reported that orally administered tetracyclines (including tetracycline, minocycline, and doxycycline) attain steady-state levels in gingival crevicular fluid (GCF) that are several times higher than their levels in serum.^{7–9} More recently, a similar distribution profile was reported for ciprofloxacin, a member of the fluoroquinolone family.¹⁰ The mechanisms by which GCF tetracycline and fluoroquinolone levels are elevated relative to serum are unclear, but previous studies have shown that gingival fibroblasts take up and accumulate tetracyclines and fluoroquinolones.¹¹ Despite a relatively low affinity of transport, the intracellular/extracellular concentration ratio observed in vitro was >60:1 for minocycline and >8:1 for ciprofloxacin. Accumulation of these agents by gingival fibroblasts could enhance their redistribution from systemic circulation to the gingiva and contribute to increased local antibiotic levels.

Since ciprofloxacin and doxycycline transporters are capable of moving their substrates in both directions in order to maintain equilibrium between intracellular and extracellular concentrations, Yang et al.¹¹ proposed that gingival fibroblasts function as reservoirs for fluoroquinolones and tetracyclines. If the redistribution of these agents involves equilibrium with fibroblasts reservoirs, their concentrations should be similar in gingival connective tissue and GCF. In the present study, we examined the redistribution of ciprofloxacin and doxycycline from blood to GCF and compared steady state levels in human serum, gingival connective tissue, and GCF after 3 days of antibiotic therapy. Our results support the hypothesis that gingival fibroblasts play an important role in the distribution of these agents to gingival connective tissue and GCF.

MATERIALS AND METHODS

Study Design

Seventeen patients were recruited from the clinic population of the Ohio State University College of Dentistry. The subjects had previously received initial periodontal therapy to minimize inflammation and had been treatment planned for resective periodontal surgery (e.g., osseous resection, crown lengthening, or gingivectomy). Pregnant females and patients taking anti-inflammatory agents, antibiotics, or any other type of medication were excluded. Informed consent was obtained under a protocol approved by the Human Subjects Institutional Review Board. The mean ages of the two groups (41.4 ± 3.8 years for the doxycycline group, $N = 8$; 45.5 ± 3.7 for the ciprofloxacin group, $N = 9$) were similar.

Patients were issued a three-day supply of ciprofloxacin or doxycycline with detailed instructions on their use. In an effort to enhance compliance with the antimicrobial regimen, subjects were asked to document the times and dates they took the medication. Prior to the surgical treatment appointment, subjects in the ciprofloxacin group took a 500 mg starting dose followed by 500 mg every 12 hours for 72 hours to establish steady-state levels in tissue and gingival crevicular fluid (GCF). Subjects in the doxycycline group took four doses of doxycycline (200 mg initially, followed by 100 mg every 24 hours for 72 hours). At the time of the surgical appointment (which was intentionally scheduled 5 to 7 hours after the final dose), GCF, blood serum, and gingival connective tissue (GCT) samples were obtained. Approximately 5 ml of peripheral blood was drawn from subjects using standard venipuncture techniques. GCF samples were then collected from 10 to 12 interproximal sites near the surgical site, using filter paper strips[†] in conjunction with a procedure previously described.¹⁰ Briefly, sites were isolated and the gingival tissues were air dried to avoid contamination with saliva.

GCF samples were collected at the orifice of the crevice for 30 seconds and measured with a gingival fluid meter[§] that had been calibrated by an established method.¹² The paper strips were pooled and stored in microcentrifuge tubes. The mean volumes of the pooled GCF samples from each subject ($1.72 \pm 0.26 \mu\text{l}$ for the doxycycline group and $1.62 \pm 0.11 \mu\text{l}$ for the ciprofloxacin group) were similar. These relatively small GCF volumes, which were pooled from 10 filter paper strip samples per subject, indicate that the sites were relatively free of inflammation in both groups.

Small samples of gingival connective tissue (GCT) were obtained during the surgical procedure from sites that had not been directly infiltrated with local anesthetic. They were blotted with saline-impregnated gauze, weighed in a microcentrifuge tube, and briefly cooled on ice. The serum, GCF, and GCT samples were stored frozen until the time of preparation for analysis by high-performance liquid chromatography (HPLC).

Sample Preparation

Ciprofloxacin and doxycycline were eluted from the GCF sample strips using a method previously described.¹³ Briefly, paper strips from each subject were pooled and eluted with two 75 μl aliquots of 100 mM glycine, pH 3. Sample recovery with this procedure was essentially quantitative. GCT samples were suspended in 200 μl sample buffer and subjected to three cycles of freezing and thawing. Samples were disrupted with disposable pestles. After precipitation of protein from the samples, the samples were evaporated under streaming nitrogen and stored in the dark at -20°C until time for HPLC analysis.

Analytical Procedures

Ciprofloxacin content in serum, GCT, and GCF was measured by HPLC, using a method described by Jim et al.¹⁴ The samples were analyzed by isocratic reversed phase chromatography on a C₁₈ column[¶] ($5 \times 100 \text{ mm}$). The mobile phase consisted of acetonitrile: 100 mM 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.

Doxycycline content in blood, tissue, and GCF was measured with the HPLC method described by Vienneau and Kindberg.¹⁵ The samples were analyzed by isocratic reversed phase chromatography on a C₁₈ column[¶] ($4.6 \times 250 \text{ mm}$). The mobile phase consisted of methanol and 0.1 M sodium acetate buffer (pH 6.5) containing 35 mM CaCl₂ and 25 mM EDTA (40:60, v/v). Sample elution was monitored with a fluorescence detector, using an excitation wavelength of 375 nm and a 480 nm long pass emission filter. Serum and GCF antibiotic levels were calculated by dividing the antibiotic content of each sample pool by its total volume. Tissue antibiotic levels were expressed as weight/weight.

RESULTS

Our previous study¹⁰ suggested that steady state ciprofloxacin levels are attained in gingiva within 28 hours of the initial dose. To gain a better understanding of the kinetics of doxycycline distribution from blood to GCF, doxycycline levels were measured in serum and GCF in a subject who had undergone 2 days of treatment with doxycycline (i.e., a 200 mg initial dose followed by 100 mg doses at 24 and 48 hours).

[‡]Periopaper, Oraflow Inc., Plainview, NY.

[§]Periotron 8000, Oraflow Inc.

[¶]Novapak, Waters Corporation, Milford, MA.

^{¶¶}Altima, Altech Corporation, Flemington, NJ.

Approximately 2 hours after the last dose, serum doxycycline levels peaked near 2.7 µg/ml and GCF levels approached 1.8 µg/ml (Fig. 1). Over the next 5 hours, serum and GCF doxycycline levels converged at a level below 2 µg/ml. Thereafter, serum levels decreased to 1.4 µg/ml while GCF levels increased above 2.2 µg/ml before falling below 1.9 µg/ml. These observations were incorporated into the design of the main study. To reduce variability and assure that measurements would accurately represent steady-state antimicrobial levels, antimicrobial agents were administered for 72 hours in the main study, and their levels in serum, GCT, and GCF were sampled 6 hours after the final dose.

To obtain evidence to support our hypothesis that fibroblasts could enhance the distribution of antibiotics to gingiva, doxycycline and ciprofloxacin levels were measured and compared in serum, GCT, and GCF (Fig. 2). Samples were obtained approximately 6 hours after the conclusion of a 72 hour course of treatment with systemic doxycycline or ciprofloxacin. Doxycycline levels averaged 1.11 ± 0.13 µg/ml, 2.03 ± 0.17 µg/g and 2.41 ± 0.39 µg/ml in serum, GCT and GCF, respectively, and were significantly different ($P = 0.002$, repeated measures analysis of variance). Levels in GCT and GCF were significantly higher than serum levels ($P < 0.05$, Tukey test), but were not significantly different from each other. Ciprofloxacin levels averaged 0.40 ± 0.035 µg/ml in serum, 1.38 ± 0.17 µg/g in GCT and 1.66 ± 0.25 µg/ml in GCF ($P < 0.001$, repeated measures analysis of variance). Again, levels in GCT and GCF were significantly higher than in blood ($P < 0.05$, Tukey test), but similar to each other.

DISCUSSION

The results support the hypothesis that fibroblasts serve as reservoirs for tetracyclines and fluoroquinolones in GCT. Steady state GCT doxycycline and ciprofloxacin levels, when measured 6 hours after ingestion and approximately 4 hours after peak serum levels, were 2.2-fold higher than serum levels for doxycycline and 4.1-fold higher than serum levels for ciprofloxacin ($P \leq 0.002$). Relative to the pronounced rise and fall of serum doxycycline and ciprofloxacin levels observed during antimicrobial chemotherapy (see Fig. 1), GCF levels appear to be less variable. By accumulating doxycycline and ciprofloxacin, fibroblasts can potentially enhance the distribution of these agents to GCT, buffer their levels at that site, and contribute to a delay between attainment of peak serum levels and attainment of peak GCF levels. In this manner, they could also contribute to the relatively high steady-state levels these agents attain in GCF.

Our previous work demonstrated that cultured gingival fibroblasts possess transporters that are capable of moving doxycycline and ciprofloxacin into or out of the cell to maintain equilibrium between intracellular and extracellular concentrations.¹¹ This equilibrium favors intracellular levels that are at least 60-fold higher than extracellular levels for minocycline and at least 8-fold higher for ciprofloxacin.¹¹ Our work demonstrated that intracellular stores of minocycline and ciprofloxacin move out of gingival fibroblasts when their concentrations decrease in the extracellular medium. During antimicrobial therapy in vivo, transport into the cell should predominate during periods in which these agents are increasing or peaking in the blood. As antibiotic levels decrease from their peak values in the blood and tissue, efflux from gingival fibroblasts could help maintain relatively high antimicrobial levels in the interstitial fluid. Due to the unique architecture of the gingiva,¹⁶ much of the antibiotic content of the interstitial fluid eventually passes through the junctional epithelium and into the gingival crevice. Assuming fibroblasts function as antibiotic reservoirs in the gingiva, this could explain why tetracycline,⁷ doxycycline,⁹ minocycline,⁸ and ciprofloxacin¹⁰ appear to reach higher levels in GCF than in blood serum when sampled after blood levels have receded from their peak values. It could also account for the findings of Sakellari et al.,¹⁷ who reported higher levels of tetracycline, doxycycline, and minocycline in serum than in GCF. Their serum and GCF samples were obtained 2 hours after oral administration, which coincides with the time of peak

serum levels. The doxycycline content of their samples (2.35 µg/ml in serum and 1.65 µg/ml in GCF) was similar to what we observed 2 hours after an oral dose of this agent (see 50-hour data points in Fig. 1).

The ciprofloxacin concentrations observed in serum and GCF in this study are similar to those previously reported by Conway et al.,¹⁰ who used identical methods of HPLC analysis. However, our observed doxycycline levels were lower than previously reported. After 48 hours of treatment with systemic doxycycline, Pascale et al.⁹ measured levels of 3 to 10 µg/ml in GCF and 2.1 to 2.9 µg/ml in blood. While the present study assayed doxycycline levels directly by HPLC, the previous study used an agar diffusion assay based on doxycycline inhibition of hemolysis by *Clostridium perfringens* grown in blood agar. This bioassay is quite sensitive,¹⁸ but cannot distinguish the antimicrobial activity of doxycycline from that of other compounds in the sample (e.g., doxycycline metabolites).

This is the first report that doxycycline and ciprofloxacin levels in gingiva can exceed those in blood serum. Since antibiotics are most useful against *A. actinomycetemcomitans*, *P. gingivalis*, and other pathogens that invade the soft tissue wall of the pocket, this finding is clinically relevant. While the doxycycline and ciprofloxacin levels observed in gingiva and GCF are much higher than their reported minimal inhibitory concentrations (MIC) for pure cultures of these two pathogens,⁴ bacteria growing in biofilms are more resistant to antimicrobial agents than planktonic cultures of the same bacteria.¹⁹ Moreover, the minimal bactericidal concentration (MBC) of most antimicrobial agents is several-fold higher than their conventional MIC value. As an example, the conventional MIC of doxycycline for most pure strains of *P. gingivalis* is around 0.125 µg/ml, but the MBC of doxycycline for *P. gingivalis* growing in biofilm is 2 to 16 µg/ml.²⁰ Thus, accumulation of tetracyclines and fluoroquinolones by fibroblasts in gingival connective tissue could potentially enhance the effectiveness of these antimicrobial agents by increasing their local concentrations in gingiva and sustaining them for a longer duration. Our findings suggest that fibroblasts play an important role in the distribution of fluoroquinolones and tetracyclines to the gingiva, which is a factor that must be considered in order to optimize periodontal antimicrobial chemotherapy.

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Figure 1.
Doxycycline levels in blood serum and GCF during the third day of systemic treatment with this agent.

Figure 2. Distribution of systemic doxycycline and ciprofloxacin in serum, gingiva, and gingival crevicular fluid. *Significantly different than serum levels ($P < 0.05$, Tukey test).