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Azithromycin Concentrations in Blood and Gingival Crevicular Fluid after Systemic Administration

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Abstract

Background—Azithromycin is a macrolide antibiotic that is active against several periodontal pathogens. Macrolides are taken up and concentrated inside gingival fibroblasts, which could influence their pharmacokinetics. This study tested the hypothesis that steady-state levels of azithromycin are higher and more sustained in gingival crevicular fluid (GCF) than in serum.

Methods—Four healthy subjects received an initial dose of 500 mg azithromycin followed by 250 mg doses on each of the next 2 days. Serum and GCF samples were obtained 2 hours after the last dose (day 2) and on days 4 and 7. GCF samples were collected from maxillary posterior sites with paper strips. The strips were pooled and eluted with high purity water. After extraction, the azithromycin content of the serum samples and GCF eluates was determined with an agar diffusion bioassay.

Results—On days 2, 4 and 7, the concentrations of azithromycin in blood serum were 0.22 ± 0.02 , 0.08 ± 0.02 and 0.04 ± 0.01 $\mu\text{g/ml}$, respectively. The concentrations in GCF were 8.82 ± 1.25 , 7.90 ± 1.72 and 7.38 ± 1.15 $\mu\text{g/ml}$, respectively. Mean GCF levels were significantly higher than mean serum levels ($P \leq 0.02$, paired t-test).

Conclusion—The findings demonstrate that the pharmacokinetic profiles of azithromycin are different in GCF and serum. At steady state, azithromycin concentrations in GCF were higher and more sustained than those in serum. Based on previous studies, the levels observed in GCF were above the MIC for *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia*.

Keywords

Anti-infective agents; Periodontitis; Tissue distribution

INTRODUCTION

Elimination of bacterial plaque from root surfaces is a major objective of periodontal therapy. Although this usually halts progression of attachment loss, this is a challenging goal in patients who are infected by invasive subgingival bacteria. *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia*, which can invade pocket epithelium,^{1–4} are particularly difficult to eliminate by scaling and root

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planing. Use of adjunctive antibiotics is a logical approach for eradicating these pathogens and enhancing the response to scaling and root planing.⁵ Azithromycin is a macrolide antibiotic that inhibits a variety of subgingival bacteria.^{6–9} Clarithromycin, a closely related macrolide, is actively transported and concentrated inside oral epithelial cells and gingival fibroblasts.¹⁰ Antibiotic uptake by host cells could provide several benefits in the treatment of periodontitis. Elevated macrolide concentrations inside oral epithelial cells could facilitate the killing of invasive pathogens. Since gingival fibroblasts are a relatively large cellular compartment of the gingiva,¹¹ macrolide accumulation by these cells could allow them to function as drug reservoirs that enhance and sustain therapeutic concentrations at that site. Previous studies have shown that azithromycin and clarithromycin can attain higher steady-state levels in gingival tissue than in serum and suggest that tissue levels are increased in the presence of inflammation.^{12,13} Azithromycin concentrations in gingiva reportedly persist for up to 14 days after systemic administration.¹⁴

Gingival crevicular fluid (GCF) originates from the vessels of the gingival plexus, within the gingival connective tissue. It seeps through gingival connective tissue and passes through the junctional epithelium prior to entering the gingival crevice.¹¹ Following systemic administration, we hypothesize that steady-state azithromycin concentrations in GCF are higher and more sustained than the corresponding concentrations in blood. The present study tested this hypothesis, using methods adapted to work with small samples of GCF.

MATERIALS AND METHODS

Subject population

Four healthy adult volunteers (3 males and one female, with a mean age of 30 years) with no clinical periodontal attachment loss were recruited from the student population of the Ohio State University College of Dentistry. Written informed consent was obtained prior to their participation. The study protocol and subject recruitment procedures were reviewed and approved by the Institutional Review Board at the Ohio State University.

Study design

One week prior to administration of azithromycin, all participants had their teeth cleaned and received oral hygiene instruction. To obtain steady-state levels of azithromycin in periodontal tissues, participants were given an initial dose of 500 mg with subsequent 250 mg doses at 24 and 48 hours. Using an established protocol,¹⁵ GCF samples were collected and pooled from twelve maxillary posterior interproximal sites (the mesiofacial and mesiolingual aspects of all maxillary first and second premolars and first molars) immediately before the first dose of azithromycin (day 0), two hours after the last dose on day 2, and on days 4 and 7 after the initial dose. These sites were selected because they were readily accessible and easy to protect from contamination by saliva. Prior to GCF collection, the collection sites were isolated with cotton rolls, supragingival plaque was removed (if present), and the site was gently dried with air. GCF was collected with paper strips[‡] positioned at the orifice of the crevice for 30 seconds. GCF volumes were determined with a calibrated gingival fluid measurement device[§] and pooled. The Gingival Index (GI)¹⁶ and Plaque Index (PI)¹⁷ were assessed at the collection sites at every study visit. In addition, blood samples were obtained by venipuncture on days 2, 4 and 7. Blood serum and pooled GCF samples were stored at -20°C in sealed vials.

[‡]Periopaper™, Oraflow, Smithtown, NY

[§]Periotron 6000, IDE Interstate, Amityville, NY

Sample analysis

Prior to analysis, GCF was eluted from the pools of paper strips with 200 μ l of ultrapure water, using a method previously described.¹⁵ The efficiency of elution, as assessed with [³H]-labeled macrolide,^{||} was 67.4% \pm 2.1% (data not shown). GCF eluates and blood serum samples (200 μ l) were treated with 40 μ l of 0.5 g/ml Na₂CO₃ and extracted three times with 1 ml diethyl ether. The extracts were dried, reconstituted in acetonitrile, and applied to sterile paper disks.[¶] After complete evaporation of the acetonitrile, the azithromycin content of the disks was determined with an agar diffusion bioassay, using *Kocuria rhizophila*[#] as the indicator organism.¹⁴ The assay was calibrated with 2 to 18 ng of authentic azithromycin.^{**} For GCF samples, calculations for azithromycin content incorporated a correction for the observed elution efficiency.

Statistical analysis

The paired t-test was used to evaluate differences in azithromycin concentration in GCF and blood. Based on a projected difference of 4 μ g/ml in the mean azithromycin concentrations in GCF and blood serum and a pooled standard deviation of 1.5, the estimated number of subjects required to achieve a power of 0.80 with an alpha of 0.05 in a paired t-test was 4. Repeated measures analysis of variance (ANOVA) was used to examine the statistical significance in changes in azithromycin concentration, GCF azithromycin content and GCF volume over the course of the study. The Holm-Sidak test was used for post-hoc comparisons. In all statistical analyses, the statistical unit was the subject rather than the site.

RESULTS

Consistent with maintenance of gingival health, the median PI and GI values were 0 throughout the course of the study (Table 1). After administration of azithromycin, there was a persistent and statistically significant reduction in the pooled volume of GCF collected from the study sites ($P < 0.001$, repeated measures ANOVA, power of test = 0.99, Figure 1A). The pooled volumes on days 2, 4 and 7 were significantly lower than the baseline level ($P < 0.05$, Holm-Sidak test). On day 2, the azithromycin concentrations in GCF and blood serum were 8.82 ± 1.25 and 0.22 ± 0.02 μ g/ml, respectively. Over the next five days, these concentrations decreased to 7.38 ± 1.15 and 0.04 ± 0.01 μ g/ml, respectively (Figure 1B). Although concentrations in GCF did not decrease significantly between day 2 and day 7, there was a significant decrease in serum concentrations on days 4 and 7 ($P < 0.05$, Holm-Sidak test). Mean GCF levels were significantly higher than mean serum levels on days 2, 4 and 7 ($P \leq 0.02$, paired t-test, power of tests ≥ 0.84). The rate of azithromycin infusion into the gingival crevice, as assessed by the amount recovered per 30 second pooled GCF sample, did not change significantly between days 2 and 7 (Table 1).

DISCUSSION

The findings support the hypothesis that steady-state azithromycin concentrations in GCF are significantly higher and more sustained than those in serum. Azithromycin concentrations in GCF were more than 40-fold higher throughout the course of the study, and they decreased at a slower rate than the levels in serum. Azithromycin levels in GCF decreased by approximately 20% between the second and seventh days, while the levels in serum decreased by 80% during the same period. This may be attributed to the low degree of

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[#]ATCC 9341, American Type Culture Collection, Manassas, VA

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azithromycin binding to plasma proteins in combination with active accumulation of azithromycin by cells in peripheral tissues.^{18,19} Gingival connective tissue contains a large volume of fibroblasts, which could serve as reservoirs for maintaining high local levels of azithromycin. Oral epithelial cells and polymorphonuclear leukocytes may also accumulate azithromycin at this site.^{10, 20} Our findings are consistent with a previous report that azithromycin concentrations in gingival tissue are up to 25-fold higher than the corresponding concentrations in blood.²¹ Macrolides are not the only antimicrobial agents that have a propensity to concentrate in GCF. Tetracyclines (e.g., doxycycline) and fluoroquinolones (e.g., ciprofloxacin) reportedly attain GCF concentrations that are several-fold higher than their concentrations in serum.^{15, 22–24} The pharmacological properties of all these agents are presumably influenced by their ability to be taken up, sequestered and released by fibroblasts, leukocytes, and other types of cells.^{10, 20}

Azithromycin concentrations observed in GCF are substantially higher than the minimal inhibitory concentration (MIC) previously reported for several periodontal pathogens, including *Aggregatibacter actinomycetemcomitans* (0.25–2.0 µg/ml),⁶ *Porphyromonas gingivalis* (0.125–1 µg/ml),^{7, 8} *Prevotella intermedia* (0.03–1 µg/ml), and *Peptostreptococcus micros* (0.5–1 µg/ml).⁸ Moreover, azithromycin concentrations remained above the MICs for these pathogens over the entire five day observation period. While many antibiotics can be classified as having either a concentration dependent killing effect or a time dependent killing effect, bacterial killing by azithromycin is not solely dependent on either model.²⁵ The time that target organisms are exposed to azithromycin concentrations above the MIC appears to be the best index of efficacy.²⁶ Azithromycin also exhibits a prolonged post-antibiotic effect on inhibition of bacterial regrowth.²⁶ Thus, pathogens found within periodontal tissues or in periodontal pockets that have been treated to disrupt subgingival biofilm should be vulnerable to inhibition by azithromycin. Pathogens living in native subgingival biofilm could be more difficult to inhibit at the azithromycin concentrations observed in this study. However, studies with an in vitro periodontal biofilm model suggest that azithromycin can penetrate the biofilm surface and partially dissolve the biofilm.²⁷ Azithromycin also appears to dissolve biofilm associated with diffuse panbronchiolitis.²⁸

A limited number of randomized, placebo-controlled clinical trials have suggested that azithromycin is a useful adjunct to scaling and root planing (SRP) in the treatment of periodontitis. In a study of patients with aggressive periodontitis, the combination of SRP plus azithromycin resulted in a higher percentage of teeth with attachment gain ≥ 1 mm and a greater reduction in probing depths than SRP alone.²⁹ In patients with chronic periodontitis, the combination of SRP plus azithromycin yielded a significantly greater reduction of probing depths for pockets initially ≥ 4 mm than SRP alone.³⁰ A later study of non-surgical treatment of chronic periodontitis in smokers also demonstrated that adjunctive use of azithromycin with SRP resulted in enhanced pocket depth reduction and clinical attachment gain at moderate (4 to 6 mm) and deep (>6 mm) periodontal sites when compared to SRP alone.³¹ Moreover, a recent study of subjects with *Porphyromonas gingivalis*-associated chronic periodontitis demonstrated that, when compared with SRP alone, SRP combined with azithromycin yielded significantly enhanced pocket depth reduction and attachment gain and a significant decrease in the detection of *Porphyromonas gingivalis*.³²

Due to their anti-inflammatory activity, macrolides have been used in immunomodulatory therapy for chronic inflammatory lung diseases.³³ Azithromycin also appears to produce anti-inflammatory effects in gingiva, as evidenced by its ability to reduce GCF volume and the GCF content of pro-inflammatory cytokines IL-1 β , IL-8 and TNF- α .³⁴ The mechanism for these effects appears to involve modulation of nuclear factor- κ B (NF- κ B) and activator

protein-1 (AP-1).³⁵ Since GCF volume is strongly correlated with histological signs of gingival inflammation,³⁶ the significant reduction of GCF volume observed between days 2 and 7 in this study is consistent with the previous report. The apparent anti-inflammatory effects of azithromycin could represent an additional benefit when applied to treatment of inflammatory periodontal diseases. In conclusion, the results demonstrate that systemic administration of azithromycin produces relatively high and sustained levels in GCF and provide a rationale for further clinical evaluation of its adjunctive benefits in the treatment of periodontitis.

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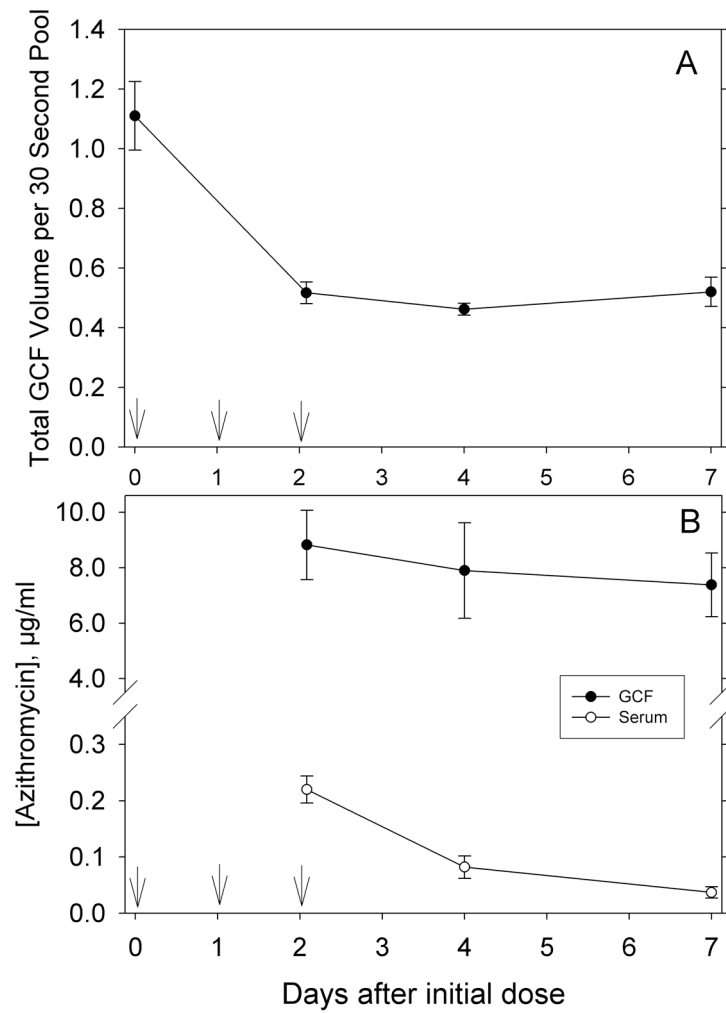


Figure 1. Changes in GCF volume and azithromycin concentrations observed during the study. Vertical arrows indicate times when azithromycin was administered. A: Gingival crevicular fluid volumes collected during the study. The data represent the mean (\pm SEM) pooled GCF volume collected for 30 seconds from 12 maxillary premolar and first molar sites. B: Azithromycin concentrations in GCF and blood serum. The mean concentrations observed in GCF were significantly higher than those in serum on days 2, 4 and 7 ($P \leq 0.02$, paired t-test).

Table 1

Clinical and Pharmacological Observations During the Study

Days After Initial Azithromycin Dose	Gingival Index*	Plaque Index*	GCF Azithromycin Content (ng per 30 second pooled sample) [†]
0	0 (0 to 0)	0 (0 to 0)	Not determined
2	0 (0 to 0)	0 (0 to 0)	3.63 ± 0.39
4	0 (0 to 0)	0 (0 to 0)	3.41 ± 0.53
7	0 (0 to 0)	0 (0 to 0)	3.26 ± 0.56

Data are presented as *median and range observed in 4 subjects or [†]mean ± SEM. Within each column, there were no statistically significant differences between the observed values.