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Effect of Gingivitis on Azithromycin Concentrations in Gingival Crevicular Fluid

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Abstract

Aim—Macrolide antibiotics yield high concentrations in inflamed tissue, suggesting that their levels in gingival crevicular fluid (GCF) could be increased at gingivitis sites. However, the increased volume of GCF associated with gingivitis could potentially dilute macrolides. To determine whether these assumptions are correct, the bioavailability of systemically-administered azithromycin was compared in GCF from healthy and gingivitis sites.

Materials and methods—Experimental gingivitis was induced in one maxillary posterior sextant in nine healthy subjects. Contralateral healthy sextants served as controls. Subjects ingested 500 mg of azithromycin followed by a 250 mg dose 24 hours later. Four hours after the second dose, plaque was removed from experimental sites. GCF was collected from 8 surfaces in both the experimental and control sextants and pooled separately. GCF samples were subsequently collected on the 2nd, 3rd, 8th and 15th days and azithromycin content was determined by agar diffusion bioassay.

Results—On days 2 and 3, the pooled GCF volume at experimental sites was significantly higher than at control sites ($P < 0.01$) and the total azithromycin mass in 30 second GCF samples pooled from experimental sites was significantly higher than at control sites ($P < 0.02$). However, there were no significant differences in azithromycin concentration between the experimental and control pools at any point. Concentrations exceeded 7.3 $\mu\text{g/ml}$ on day 2 and 2.5 $\mu\text{g/ml}$ on day 15.

Conclusions—Azithromycin concentrations are similar in GCF from gingivitis sites and healthy sites, suggesting that the processes that regulate GCF azithromycin concentration can compensate for local inflammatory changes.

Keywords

Anti-infective agents; inflammation; pharmacokinetics

INTRODUCTION

Aggregatibacter actinomycetemcomitans and *Porphyromonas gingivalis* are periodontitis-associated bacteria that are capable of invading cells in the soft tissue wall of periodontal pockets.^{1, 2} These invasive pathogens are difficult to eliminate by mechanical debridement

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alone,³ but adjunctive use of systemic antibiotics can enhance the therapeutic response to scaling and root planing (SRP).^{4, 5} As an example, azithromycin is a macrolide antibiotic that is active against *A. actinomycetemcomitans*, *P. gingivalis*, and other Gram negative anaerobes.^{6–8} Randomized clinical trials suggest that azithromycin enhances clinical attachment gain and pocket reduction in response to SRP in patients with aggressive and chronic forms of periodontitis.^{9–12} Adjunctive azithromycin also reduces the frequency of *P. gingivalis* detection after SRP in patients with chronic periodontitis.¹²

The pharmacokinetic properties of macrolides are influenced by their tendency to be taken up and released by many types of host cells. Macrolides accumulate at high levels inside phagocytes^{13, 14} and are actively transported by fibroblasts and oral epithelial cells.^{15, 16} The existence of these “macrolide reservoirs” may allow azithromycin and clarithromycin to attain high steady-state levels in gingiva.^{17, 18} It may also explain why steady-state concentrations of azithromycin are significantly higher and more sustained in gingival crevicular fluid (GCF) than in blood serum.¹⁹

While previous studies have shown that the azithromycin content of periodontal tissues tends to increase in the presence of inflammation,^{17, 18} it is unclear whether inflammation is accompanied by increased macrolide levels in GCF. Although antibiotics are not used to treat gingivitis, experimental gingivitis is a well-characterized, reversible model of inflammation that exhibits characteristic features that can potentially influence drug levels in GCF. Gingivitis is associated with an increase in the volumetric density of fibroblasts and phagocytes in gingival connective tissue,²⁰ increasing the local capacity for macrolide accumulation. Gingivitis is also accompanied by increased vascular and junctional epithelial permeability and a significant increase in the volume of interstitial fluid and GCF.^{21, 22} This could potentially elute macrolides from intracellular storage sites and, ultimately, dilute their levels in interstitial fluid and GCF. The purpose of this pharmacokinetic study was to assess the influence of gingivitis on azithromycin distribution to the gingival crevice and its impact on therapeutic concentrations of azithromycin in GCF.

MATERIALS AND METHODS

Subject selection

Approval of this study was obtained from the Office of Responsible Research Practices at The Ohio State University. Healthy adult nonsmokers (3 males/6 females, mean age 30.4 years) in good systemic and periodontal health were recruited from the student population of the Ohio State University. Written informed consent was obtained from all subjects, using procedures approved by the Institutional Review Board. Subjects exhibited no evidence of clinical attachment loss, aside from localized minor facial gingival recession. Subjects with a history of drug allergy or recent medication use were excluded, as were pregnant females.

Study Design

Table 1 provides an overview of the timeline for this split-mouth prospective study. One week prior to a baseline periodontal examination, each subject was treated with a supragingival prophylaxis. A thermoplastic stent was fabricated to cover the maxillary posterior teeth on one randomly selected side. At the baseline examination, Gingival Index (GI)²³ and Plaque Index (PI)²⁴ were measured on the mesiobuccal and mesiolingual aspects of the maxillary premolars and molars. GCF samples were collected and pooled from the same eight posterior sites on each side as previously described.²⁵ Briefly, sites were scaled to remove supragingival plaque, isolated with cotton rolls, and air dried to avoid contamination. GCF samples were then collected by positioning a paper strip[‡] at the orifice

of the sulcus for 30 seconds. GCF volume was measured with a calibrated gingival fluid measurement device[§]. The eight samples from each side were pooled and stored at -20°C .

After the baseline examination, subjects were given a thermoplastic stent to facilitate induction of experimental gingivitis in one posterior region of the maxilla.²⁶ Subjects were instructed to wear the stent during routine oral hygiene procedures to prevent plaque removal from the experimental side, but were expected to maintain good oral hygiene at all other sites. Posterior sites in the contralateral quadrant served as healthy controls.

The subjects returned every seven days for the next three weeks for assessment of GI and PI at the control and experimental sites. At the three week visit, GCF samples were collected from the control sites. On the following day (Day 0), the subjects were administered an initial oral dose of 500 mg azithromycin followed by a 250 mg dose 24 hours later. Four to six hours later after the second dose, GI and PI were assessed at the control and experimental sites and plaque was gently removed from the experimental teeth. GCF samples were collected from the control and experimental sites, measured and pooled separately as previously described. A sample of peripheral blood was obtained by venipuncture. The subjects were instructed to immediately resume normal oral hygiene procedures. GI and PI assessments and GCF and blood samples were obtained in the same manner on days 2, 3, 8 and 15 after the initial dose of azithromycin.

To enhance objectivity and reduce delays that could lead to sample contamination or evaporation, GCF samples were collected by a team that included a clinical operator (NJ), a GCF measurement device operator (JW) and a data recorder (PL). The clinical operator assessed the PI and GI scores, isolated the GCF collection sites and collected GCF samples. The GCF measurement device operator measured the sample volumes and stored the samples in a cooled microtube. The GCF measurement device readout was not visible to the clinical operator during sample collection and the site of origin of the GCF sample was not visible to the GCF measurement device operator. Only the data recorder had direct access to this information.

Sample preparation and analysis

Prior to analysis, GCF was eluted from each pool of paper strips with 200 μl of liquid chromatography grade water, using a method previously described.²⁵ The efficiency of elution, as assessed with [³H]-labeled macrolide^{||}, was 67.4%. GCF eluates and blood serum samples (200 μl) were treated with 40 μl of 0.5 g/ml Na_2CO_3 and extracted three times with 1 ml diethyl ether. The extracts were dried under streaming nitrogen, reconstituted in acetonitrile, and applied to sterile paper disks[¶]. After evaporation of acetonitrile, the azithromycin content of the disks was determined with an agar diffusion bioassay, using *Kocuria rhizophila*[#] as the indicator organism.¹⁸ The assay had a detection limit of 1 ng and an inter-assay coefficient of variability of <9% in calibrations with authentic azithromycin^{**}. The reported azithromycin content of GCF incorporated a correction for the efficiency of elution.

‡Periopaper™, Oraflow, Smithtown, NY

§Periotron 6000, IDE Interstate, Amityville, NY

||American Radiolabeled Chemicals, St. Louis, MO

¶BD Biosciences, Sparks, MD

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

**US Pharmacopeia, Rockville, MD

RESULTS

At the baseline examination (22 days prior to the first dose of azithromycin), median PI and GI values at the control and experimental sites were 0 (Figure 1), and the mean volumes of pooled GCF samples from these sites were 0.98 μ l and 0.88 μ l, respectively (Figure 2). There were no significant differences between control and experimental sites.

During induction of experimental gingivitis, PI and GI values did not change at the control sites. At experimental sites, both indices increased to a median value of 1.5 within 21 days. With respect to PI and GI, the differences between control and experimental sites were significant at every observation point during this period ($P = 0.016$, Wilcoxon signed-rank test). After initiation of the azithromycin regimen, PI and GI values decreased at experimental sites on day 1 (Figure 1). The decrease in PI was statistically significant ($P = 0.019$, Mann-Whitney rank sum test). GI decreased to baseline level within 7 days after plaque was removed from the experimental sites.

At control sites, there were no significant changes in pooled GCF volume during the period of gingivitis induction at experimental sites (Figure 2). In contrast, the GCF volume at experimental sites increased by more than two-fold. GCF volumes at experimental sites were significantly higher than control on days 1 through 3 after the initial azithromycin dose ($P = 0.01$, paired t-test), although the volume at experimental sites decreased by approximately 40% between days 1 and 3. GCF volumes at control and experimental sites converged after day 3 and were not significantly different on days 8 and 15. Interestingly, there was a transient 30% decrease in mean control GCF volume between days 1 and 3. On day 15, however, the observed volumes were similar to those observed prior to administration of azithromycin.

The total azithromycin mass in 30 second GCF samples pooled from the experimental sites was consistently higher than control from day 1 to day 3 (Figure 3). The differences were significant on days 2 and 3 ($P = 0.02$, paired t-test). Thereafter, the total azithromycin mass in experimental and control samples was not significantly different. Between day 1 and day 15, the total azithromycin mass in the experimental samples was closely correlated with GCF volume at the same sites, but was not significantly correlated with serum azithromycin concentration (Table 2). Over the same time interval, the total azithromycin mass in control samples was closely correlated with serum azithromycin concentration, but not with GCF volume.

On day 1, the azithromycin concentration in control GCF was higher than in experimental GCF (Figure 4). Thereafter, the concentrations at control and experimental sites converged and were nearly identical on days 8 and 15. While there were no significant concentration differences between control and experimental sites between day 1 and day 15, azithromycin levels in GCF was consistently higher than in blood ($P < 0.011$, repeated measures ANOVA, $P < 0.05$, Holm-Sidak test). The azithromycin concentration in control GCF was approximately 26-fold higher than in serum on day 1 and 560-fold higher on day 15. Between days 1 and 15, GCF azithromycin concentration at experimental sites did not exhibit a strong relationship with GCF volume or serum azithromycin concentration (Table 2). At control sites, GCF azithromycin concentration was not well correlated with GCF volume, but exhibited a significant correlation with serum azithromycin concentration.

DISCUSSION

The findings of this study demonstrate that azithromycin concentrations are not significantly different in GCF from gingivitis sites and healthy sites. Although the mass flow rate of azithromycin entering inflamed gingival crevices was almost twice that observed at healthy

sites, the volume of GCF passing through crevices affected by gingivitis was also twice that measured in healthy crevices. Azithromycin concentrations were 15 to 50-fold higher in GCF than in blood serum on days 1 and 2 and >50-fold higher at later time points. This suggests that the processes that regulate azithromycin concentration in GCF maintain a relatively narrow concentration range that is surprisingly independent of the degree of local inflammation.

Drug concentrations in GCF can potentially be influenced by several factors associated with gingivitis. Vasodilatation increases the amount of drug distributed to the vessels of the gingival plexus, from which GCF originates.²¹ Leakiness of vessels in the gingival plexus increases movement of drug from blood to interstitial fluid and also increases the volume of interstitial fluid. Increased permeability of the junctional epithelium allows an increased volume of interstitial fluid to enter the crevice. In the initial stage of experimental gingivitis, there is alteration of the junctional epithelium and gingival vessels exhibit vasculitis, loss of perivascular collagen, and increased neutrophilic migration out of the vessels.²² These are accompanied by increased vascular and junctional epithelial permeability. Drugs that enter the interstitial fluid seep through gingival connective tissue and eventually cross the junctional epithelium into the gingival crevice. However, a substantial amount of macrolide antibiotics may be taken up from interstitial fluid and concentrated inside fibroblasts, epithelial, inflammatory and immune cells.¹³⁻¹⁶ These cells account for a relatively large specific volume of gingiva in health and an even larger volume in the presence of gingivitis.²⁰ Since these cells can serve as macrolide reservoirs, they are thought to enhance macrolide distribution to gingiva and account for the large concentration difference between blood serum and GCF.¹⁹

These factors may also account for the differences observed between gingivitis and control sites with respect to the amount of azithromycin recovered from pooled 30 second GCF samples. At gingivitis sites, the amount of azithromycin recovered was initially larger than at control sites and changes over time were strongly correlated with changes in GCF volume. At control sites, which presumably exhibited normal vascular and junctional epithelial permeability, there was a lower azithromycin content in GCF and changes over time correlated with changes in serum azithromycin concentration. As gingivitis resolved, differences in vascular and junctional epithelial permeability presumably disappeared and the azithromycin content of control and experimental GCF were very similar on days 8 and 15.

In this study, two doses of azithromycin (750 mg total) produced sustained and relatively high antimicrobial concentrations in GCF. Two weeks after the final dose, GCF azithromycin concentrations were above the minimal inhibitory concentrations for *A. actinomycetemcomitans* (0.25 – 2.0 µg/ml), *P. gingivalis* (0.125 – 1 µg/ml) and *Prevotella intermedia* (0.03 – 1 µg/ml).⁶⁻⁸ Azithromycin exhibits time-dependent killing activity. Its antimicrobial effects are more dependent on the amount of time it is in contact with bacteria than its concentration at the infection site. The efficacy of time-dependent antibiotics can be predicted by the amount of time that the antibiotic exceeds the MIC of the target pathogens.²⁷ Assuming the observed azithromycin concentrations in GCF are representative of concentrations in gingival connective tissue, the results suggest that azithromycin could produce beneficial antimicrobial activity, even in patients who fail to complete the standard 1.5 g regimen.

Subjects were administered only two doses of azithromycin in this study because of concerns that azithromycin could reduce the severity of experimental gingivitis. Azithromycin reportedly penetrates and dissolves periodontal biofilm²⁸ as well as pulmonary biofilm associated with diffuse panbronchiolitis.²⁹ Moreover, azithromycin

induces a transient decrease in GCF volume at healthy periodontal sites in parallel with transient reductions in the GCF content of IL-8, IL-1 β and TNF- α .³⁰ Some anti-inflammatory effects of azithromycin have been attributed to down-regulation of cytokine gene expression via nuclear factor- κ B and activator protein-1.³¹ There is evidence that these were reasonable concerns. Azithromycin appeared to induce a decrease in PI and GI at experimental sites that was apparent on day 1 of the study. In addition, azithromycin induced a transient decrease in pooled GCF volume at control sites. This suggests that azithromycin may have also contributed to the rapid reduction of GCF volume observed at experimental sites after termination of experimental gingivitis.

CONCLUSIONS

The mass flow rate of azithromycin is higher at gingivitis sites than at healthy sites, but there is no significant difference in concentration. It is possible that elution of azithromycin from intracellular storage sites helps maintain this relatively narrow concentration range. Although the subjects were administered only half the standard total dose of azithromycin, its concentration in GCF remained above the minimal inhibitory concentration for several periodontal pathogens for at least two weeks after the final dose. These pharmacokinetic properties, in conjunction with its antimicrobial and anti-inflammatory activities, suggest that azithromycin is well suited for treating inflammatory periodontitis.

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Figure 1.

Changes in clinical indices observed during the study. Arrows indicate the beginning and end of the plaque accumulation during the experimental gingivitis phase. A: Plaque Index at control and experimental sites. Differences between the control and experimental sites were statistically significant at all time points from day -15 to day 1 ($P < 0.05$, Wilcoxon signed rank test). B: Gingival Index at control and experimental sites. Differences between control and experimental sites were statistically significant from day -15 to day 4 ($P < 0.05$).

Figure 2.

Changes in pooled GCF volume at control and experimental sites. Each pool was derived from eight paper strip samples, with each strip collected for 30 seconds. The data are presented as mean + SEM. Arrows indicate the beginning and end of the experimental gingivitis phase. Asterisks denote significant differences between control and experimental volumes ($P < 0.05$, paired t-test).

Figure 3.

Azithromycin content of GCF samples pooled separately from control and experimental sites. Data are presented as mean + SEM, and the arrow indicates the end of experimental gingivitis phase. Asterisks denote significant differences between control and experimental pools ($P < 0.05$, paired t-test).

Figure 4.

Azithromycin concentrations in blood serum and pooled GCF samples. Data are presented as mean + SEM. The arrow denotes the time plaque was removed at the end of the experimental gingivitis phase. Azithromycin concentrations in GCF from control and experimental sites were not significantly different. However, concentrations measured in control GCF were significantly higher than those found in blood at all observation points ($P < 0.05$, repeated measures ANOVA with post-hoc Holm-Sidak test).

Table 1

Study Timeline

Day	Study Activity
-29	Supragingival prophylaxis
-22	Measure baseline PI and GI Collect baseline GCF samples Begin experimental gingivitis induction
-15, -8	Measure PI and GI
-1	Measure PI and GI Confirm presence of gingivitis Collect control GCF sample
0	Azithromycin dose #1
1	Azithromycin dose #2
1 (+ 4-6 hr)	Remove plaque from experimental sites Measure PI and GI Collect GCF and blood samples Subjects resume oral hygiene
2, 3, 8, 15	Measure PI and GI Collect GCF and blood samples

Table 2

Correlations between Azithromycin Concentration, Azithromycin Mass Flow Rate and GCF Volume at Experimental and Control Sites

Dependent variable	Independent variable	R, experimental sites	R, control sites
[azithromycin] in GCF	azithromycin in GCF (ng/30 sec)	0.896 [*]	0.990 [*]
[azithromycin] in GCF	[azithromycin] in serum	0.408	0.838
[azithromycin] in GCF	GCF volume	0.757	0.494
azithromycin in GCF (ng/30 sec)	[azithromycin] in serum	0.643	0.881 [*]
azithromycin in GCF (ng/30 sec)	GCF volume	0.966 [*]	0.597

^{*} denotes statistically significant correlations ($P < 0.05$, ANOVA)