Published in final edited form as:

J Periodontol. 2008 September ; 79(9): 1712–1718. doi:10.1902/jop.2008.080013.

Distribution of Systemic Clarithromycin to Gingiva

Renita C. Burrell* and John D. Walters*,§

*Section of Periodontology, College of Dentistry, The Ohio State University

Abstract

Background—Aggressive and recurrent forms of periodontitis are associated with infections by *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. Since these pathogens invade tissue, they are difficult to eliminate by root planing alone. Use of systemic antibiotics in conjunction with root planing significantly enhances clinical and microbiological treatment outcomes. While it is not widely prescribed by periodontists, clarithromycin is potentially useful because it is taken up by host cells and has favorable antimicrobial activity.

Methods—Experimental gingivitis was induced in eight healthy subjects at one randomly selected maxillary posterior site. The contralateral maxillary site served as the healthy control. Thereafter, subjects were administered 6 doses of 500 mg clarithromycin every 12 hours. Blood was then drawn and samples of gingiva were harvested from both sites. The samples were extracted and clarithromycin content was analyzed by liquid chromatography.

Results—Mean clarithromycin concentrations in healthy control and inflamed gingiva (2.4 µg/g and 3.0 µg/g, respectively) were significantly higher than in serum (0.5 µg/ml, $P < 0.05$). Clarithromycin levels at control and gingivitis sites were higher than serum by 5.7 and 7.0-fold, respectively (difference between sites significant, $P = 0.02$). At control sites, a significant decrease in gingival crevicular fluid flow rate was evident at the conclusion of the clarithromycin regimen ($P = 0.018$).

Conclusions—Clarithromycin can attain higher levels in gingiva than serum and reaches higher levels in inflamed gingiva than in healthy gingiva. Its distribution profile appears to be suitable for treatment of periodontitis. The reduction in crevicular fluid flow at control sites suggests that clarithromycin may produce anti-inflammatory effects.

Keywords

macrolides; antimicrobial chemotherapy; inflammation; aggressive periodontitis

A major focus of conventional periodontal therapy is debridement of bacterial plaque and removal of bacterial products from root surfaces. While this approach usually arrests periodontal attachment loss, periodontal breakdown continues in some individuals despite careful treatment. This is often related to persistent infection by invasive subgingival bacteria. The periodontal pathogens *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* can resist debridement and evade the host response by invading epithelial cells lining the gingival crevice. One logical approach for eliminating these pathogens is to target them with antibiotics. The use of systemic antibiotics in conjunction with scaling and root planing significantly enhances gains in clinical attachment level in comparison to treatment with scaling and root planing alone.^{1,2}

§Address correspondence and reprint requests to: John D. Walters., College of Dentistry, The Ohio State University, 305 West 12th Avenue, Columbus, OH 43210., Telephone: (614) 292-1169, Fax: (614) 292-2438 (can be published), email: walters.2@osu.edu (can be published).

While not widely used in periodontal therapy, clarithromycin possesses a broad antimicrobial spectrum, favorable tissue distribution, and a low incidence of adverse side effects.³ Unlike many antibiotics, clarithromycin readily penetrates cells to gain access to intracellular pathogens. A related drug, azithromycin, can improve non-surgical periodontal therapy outcomes in patients with chronic periodontitis in clinical trials.^{4,5} Clarithromycin and azithromycin are macrolide antibiotics that inhibit protein synthesis by binding to the 23S ribosomal RNA in the 50S subunit of the bacterial ribosome.⁶ Both agents are highly effective against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*,^{7–10} and both exhibit good activity against *Eikenella corrodens*, *Prevotella* species, fusobacteria and other anaerobic and facultative pathogens.^{10–12} While clarithromycin has not been evaluated in clinical trials, it is potentially more effective against *Porphyromonas gingivalis* and *Prevotella intermedia* than azithromycin¹⁰ and inhibits *Aggregatibacter actinomycetemcomitans* with similar potency.^{7,9}

Macrolides attain high therapeutic concentrations in tissue,¹³ so they are potentially a good choice for inhibiting invasive periodontal pathogens. In-vitro studies have shown that many types of cells accumulate high intracellular levels of clarithromycin, suggesting that the agent is actively transported.¹⁴ Gingival fibroblasts and oral epithelial cells are capable of accumulating intracellular clarithromycin levels that are higher than extracellular levels by 38-fold and 3.3-fold, respectively.¹⁵ This suggests that fibroblasts could serve as reservoirs for clarithromycin in gingival connective tissue

Previous studies have suggested that macrolides reach significantly higher concentrations in infected tissue in comparison to healthy tissue.^{16,17} Clarithromycin is accumulated by phagocytes,^{18–21} monocytes,^{22,23} fibroblasts,^{15,24} polymorphonuclear cells, macrophages, and lymphocytes.¹⁴ Since those cells are more numerous at gingivitis sites, it is reasonable to expect clarithromycin levels to be higher at inflamed sites than at healthy sites. The purpose of this study was to determine whether the distribution profile of clarithromycin is suitable for treatment of periodontitis. Our hypotheses are that clarithromycin can reach significantly higher concentrations in gingival tissue than in serum, and that its levels in inflamed gingiva are significantly higher than those in healthy gingiva.

MATERIALS AND METHODS

Subjects

Eight healthy nonsmoking adult subjects with no clinical periodontal attachment loss and probing depths of 2 to 3 mm were recruited from the clinic population of the Ohio State University College of Dentistry between October, 2006 and May, 2007. Subjects who were pregnant, lactating or had a history of a drug allergy, systemic disease or recent use of other drugs were excluded from the study. All subjects had a maxillary premolar and first molar with interproximal contact on the right and left sides. Informed consent was obtained under a procedure approved by the Institutional Review Board for research involving human subjects.

Study Design

The design was a split mouth experimental prospective longitudinal cohort study. In a pre-experimental phase, subjects were prepared for the experimental gingivitis model. All patients received oral hygiene instruction and a prophylaxis and were monitored until gingival health was attained. Gingival health was assessed using the Gingival Index (GI25), Plaque Index (PI26) and gingival crevicular fluid (GCF) flow rate. GI and PI were recorded for 4 surfaces (distobuccal, distolingual, mesiobuccal, and mesiolingual of the interproximal space) of the maxillary premolar and first molar for each side. GCF samples were collected on the buccal and lingual surfaces of these teeth with filter paper strips.* The sites were lightly scaled to

remove supragingival plaque and isolated with cotton rolls. Gingival tissues were gently air dried to avoid contamination with saliva. GCF samples were collected by positioning a paper strip at the orifice of the sulcus for 45 seconds (a collection interval that consistently yielded measurable sample volumes at healthy and gingivitis sites). GCF flow rate was determined with a device for measuring the volume of collected samples,[†] which was calibrated by an established method.²⁷ A thermoplastic stent covering the maxillary premolar and first molar of one randomly selected side was fabricated.

In the second phase, which commenced one week after attainment of gingival health, experimental gingivitis was induced at one randomly selected maxillary posterior site as described by Loe et al.²⁸ The opposite posterior site remained healthy and served as the control. Baseline GI, PI, and GCF flow rate measurements were measured at the control and experimental gingivitis sites. The maxillary stent was seated on the subject's teeth. Subjects were instructed to continue routine oral hygiene procedures at all sites except the one covered by the stent. The stent was worn during routine oral hygiene procedures to prevent plaque removal from the experimental gingivitis site. Subjects returned after 7, 14, and 21 days for assessment of GI and PI at the control and experimental gingivitis sites. Clinical evidence of gingivitis at the experimental site was noted within 21 days and was indicated by an increase in GI from 0 to 1.

Immediately after the induction of experimental gingivitis, subjects were placed on a regimen consisting of 500 mg clarithromycin every 12 hours for a total of 6 doses. Six to eight hours after the last dose, GI and PI were measured at the experimental gingivitis and control sites. After light scaling to remove supragingival plaque, GCF flow rate was also measured. The greater palatine nerve was anesthetized at the control and experimental gingivitis sites by infiltration distal to the biopsy sites with 3% mepivacaine hydrochloride. Samples of palatal interproximal gingiva (25 to 60 mg wet weight) were obtained from the control and experimental sites with a sterile scalpel. Tissue samples were rapidly blotted with saline-impregnated gauze, transported to the laboratory on ice, weighed, and frozen. Hemostasis was obtained by direct pressure on the biopsy sites. A 4 ml sample of peripheral blood was obtained by venipuncture, allowed to clot, and centrifuged for collection of serum. The tissue and serum samples were stored frozen until the time of analysis. The subjects' teeth were cleaned and normal oral hygiene measures were immediately resumed. Subjects returned within 1 month to confirm that the biopsy sites had healed properly, gingivitis had resolved, and probing depths were identical to baseline readings.

Sample preparation and analysis

Serum samples were subjected to two extraction steps with diethyl ether. Tissue samples were subjected to three cycles of freezing and thawing, macerated with a micro-pestle, and extracted twice with diethyl ether. The organic phase from the extracts were evaporated under nitrogen and prepared for analysis as described by Torano and Guchelaar.²⁹ The samples were derivatized with 9-fluorenylmethyloxycarbonyl chloride and subjected to isocratic reverse-phase high performance liquid chromatography as previously described.²⁹ Samples were chromatographed on a base-deactivated C₁₈ column[§] (4.6 × 150 mm). The mobile phase consisted of acetonitrile: 0.05M potassium dihydrogen phosphate and 500 µl triethylamine adjusted to pH 7.5 (62:38 v/v). The assay was calibrated with a clarithromycin reference standard.[¶] Serum clarithromycin concentrations were expressed as µg/ml, while concentrations in gingiva were expressed as µg/g wet tissue weight.

Statistical analysis

Differences in PI and GI at control and experimental gingivitis sites were analyzed by the Wilcoxon Signed Rank test. GCF flow rate data were tested to confirm normality and equal

variance. Differences in flow rate were analyzed with the paired *t* test. Clarithromycin concentrations in serum, healthy tissue and inflamed tissue were analyzed with one way repeated measures analysis of variance after testing confirmed normality and equal variance. All-pairwise post hoc comparisons were conducted with the Holm-Sidak test.

RESULTS

Clinical findings

At baseline (day 0), median GI and PI values were 0.0 at both the control and the experimental gingivitis sites (Table 1 and 2). Mean GCF flow per 45 seconds was 0.134 ± 0.020 μ l at control sites and 0.152 ± 0.017 μ l at gingivitis sites (Table 3). There were no significant differences between control and experimental gingivitis sites with respect to any of these values.

On days 7 and 14, the GI at control sites was unchanged. However, at experimental gingivitis sites, GI increased to 1.0 by day 7 and 1.25 by day 14 at experimental gingivitis sites (Table 1). PI at control sites increased to 0.125 by day 7, but reverted to 0.0 by day 14. PI at experimental gingivitis sites increased to 1.25 by day 7 and 1.625 by day 14 (Table 2). The differences between control and experimental gingivitis sites were significant for PI and GI on days 7 and 14 ($P = 0.008$, Wilcoxon Signed Rank test).

After gingivitis was established (day 21), GI values at control and experimental gingivitis sites increased to 0.125 and 1.75, respectively (Table 1). PI at control sites was unchanged, but increased to 2.0 at gingivitis sites (Table 2). The differences between control and gingivitis sites were statistically significant ($P = 0.008$, Wilcoxon Signed Rank test).

Administration of clarithromycin between days 21 and 24 did not induce any change in GI at control or gingivitis sites (Table 1), and there was no significant change in PI at the control sites (Table 2). There was a reduction in PI at gingivitis sites, but site PI remained significantly higher than that of the control sites ($P = 0.008$, Wilcoxon Signed Rank test). Mean GCF flow rate after administration of clarithromycin decreased to 0.044 ± 0.010 μ l per 45 seconds at control sites and increased to 0.232 ± 0.023 μ l at gingivitis sites (Table 3). Compared to baseline, the decrease in flow rate at control sites and the increase in flow rate at experimental gingivitis sites were statistically significant ($P = 0.015$ and $P = 0.018$, respectively, paired *t* test).

Pharmacological findings

Approximately six hours after the last dose of clarithromycin, mean clarithromycin concentrations in serum, healthy gingiva and inflamed gingiva were 0.5 μ g/ml, 2.4 μ g/g and 3.0 μ g/g, respectively (Table 4). The concentration differences observed between these three compartments were significant ($P < 0.001$, repeated measures ANOVA). In particular, clarithromycin concentrations in healthy and inflamed tissue were both significantly higher than in serum ($P < 0.05$, Holm-Sidak test). The results were stratified to account for differences in serum clarithromycin concentration. In the four subjects with serum clarithromycin concentrations of less than 0.4 μ g/ml (mean 0.37 μ g/ml), the mean concentrations in healthy and inflamed tissue were relatively high at 3.02 μ g/ml and 3.71 μ g/g, respectively. In this subgroup, all pairwise comparisons between serum, healthy tissue and inflamed tissue revealed significant differences ($P < 0.05$, Holm-Sidak test). In the four subjects with serum clarithromycin concentrations of greater than 0.4 μ g/ml (mean 0.64 μ g/ml), the mean concentrations in healthy and inflamed tissue were relatively low at 1.78 μ g/g and 2.29 μ g/g, respectively. In this subgroup, concentrations in healthy and inflamed tissue were significantly higher than in serum ($P < 0.05$, Holm-Sidak test), but were not significantly different from each other. Consistent with these findings, the ratio of tissue concentration to serum concentration

was highest in the subgroup with lower mean serum clarithromycin concentrations and lowest in the subgroup with relatively high serum clarithromycin concentrations (Table 5). In the latter subgroup, the gingivitis site tissue/serum concentration ratio was higher, but not significantly different from, the control site gingiva/serum concentration ratio ($P > 0.3$, paired t test).

DISCUSSION

The results of this study suggest that systemically-administered clarithromycin is preferentially distributed to inflamed sites. The mean steady-state clarithromycin concentration observed in tissue from experimental gingivitis sites was 25% higher than that found in healthy control tissue. Moreover, the ratio of tissue clarithromycin concentration to serum concentration was significantly higher in inflamed gingiva than in healthy gingiva. The findings are consistent with a previous report that azithromycin attains significantly higher levels in chronically inflamed periodontal tissues than in healthy gingiva.³⁰ The data exhibited a moderate degree of subject-to-subject variability. Individual subjects could be stratified into two groups according to their serum clarithromycin levels. The group with lower serum clarithromycin levels exhibited higher tissue levels. It is likely that the two groups differed with regard to the degree of drug redistribution at the time tissue and blood samples were collected. A similar pattern of distribution reportedly occurs with ciprofloxacin and doxycycline.³¹ Clarithromycin levels in serum and tissue can be influenced by ingestion of food. Co-ingestion of food with clarithromycin can increase the bioavailability of clarithromycin by 25% or more.³²

While the main objective of this study was to determine whether clarithromycin attains effective antimicrobial levels in gingiva, there are reports that some of the therapeutic benefits attributed to macrolides are related to their anti-inflammatory properties.¹⁶ When used to treat airway and pulmonary infections, macrolides reduce inflammation by inhibiting expression of the pro-inflammatory cytokines IL-1 β , IL-6 and IL-8³³ (which enhance cell recruitment to inflammatory sites). Macrolides also inhibit neutrophil superoxide production, phagocytosis, adhesion, and chemotaxis and increase neutrophil down-regulation by apoptosis.^{16,34} In light of these effects, it was interesting to observe a significant decrease in the GCF flow rate at control sites at the conclusion of the clarithromycin regimen (day 24, Table 3). While this decrease in GCF flow could be attributable to anti-inflammatory effects produced by clarithromycin, the design of this study did not provide definitive evidence. To avoid interference with the formation of plaque and the induction of gingivitis at experimental sites, we did not attempt any measurements of GCF flow between baseline and day 24. Without these measurements, it is difficult to directly correlate administration of clarithromycin to incremental changes in GCF flow between day 21 and day 24.

For a similar reason, it was not possible to determine whether clarithromycin produced anti-inflammatory effects at the experimental gingivitis sites between days 21 and 24. GCF flow rate at gingivitis sites increased by 52% between baseline and day 24, a change which is similar in magnitude to that reported in a previous study.³⁵ The Plaque Index at experimental gingivitis sites decreased somewhat from day 21 to day 24. The study did not measure changes in GCF flow at these sites over the same period, but Gingival Index did not appear to change. This suggests that clarithromycin may not have produced anti-inflammatory effects at the gingivitis sites. In contrast to the situation at plaque-free control sites, however, it is possible that clarithromycin was not administered long enough to produce significant anti-inflammatory effects in the presence of plaque-induced gingivitis. Inhibition of bacteria in a biofilm is by nature a time-dependent process, and bacteria produce pro-inflammatory stimuli for as long as they are present. Unfortunately, the study did not ascertain whether the anti-inflammatory effects of clarithromycin could ultimately prevail at gingivitis site if the agent were administered for a longer period. Since the study was designed to assess steady-state

clarithromycin levels in healthy and inflamed gingiva, clarithromycin was administered just long enough to assure that it reached steady-state levels in tissue (3 days).

In summary, clarithromycin appears to possess pharmacological and antimicrobial properties that are appropriate for adjunctive use in the treatment of periodontitis. It accumulates in gingival tissue at levels that are significantly higher than those in serum, and appears to be preferentially distributed to inflamed periodontal sites. Clarithromycin reportedly inhibits *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and several other periodontal pathogens at concentrations that are much lower than the steady-state levels we observed in gingiva.^{9,10} Moreover, there is a suggestion that clarithromycin may produce anti-inflammatory effects in the gingiva. When prescribing any antibiotic, the balance between potential benefits and adverse side effects must be considered. The side effects of clarithromycin are typically mild, but there are reports of allergic reactions, gastrointestinal disturbances and hepatic reactions in a small number of patients.³⁶ On balance, however, the pharmacological antimicrobial, and potential anti-inflammatory properties of clarithromycin make it worthy of evaluation in clinical trials for the treatment of periodontitis.

Acknowledgements

The authors are grateful for the advice of Drs. Dimitris Tatakis and Mike Beck of the Ohio State University College of Dentistry with respect to the design of the study. This investigation was supported by USPHS grant R01 DE012601 from the National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892, USA. The authors report no financial relationships with the providers of the products used to conduct this study.

References

1. Beikler T, Prior K, Ehmke B, Flemmig TF. Specific antibiotics in the treatment of periodontitis—a proposed strategy. *J Periodontol* 2004;75:169–175. [PubMed: 15025229]
2. Slots J. Systemic antibiotics in periodontics. *J Periodontol* 2004;75:1553–1565. [PubMed: 15633334]
3. Moore PA. Dental therapeutic indications for the newer long-acting macrolide antibiotics. *J Am Dent Assoc* 1999;130:1341–1343. [PubMed: 10492541]
4. Smith SR, Foyle DM, Daniels J, et al. A double-blind placebo-controlled trial of azithromycin as an adjunct to non-surgical treatment of periodontitis in adults: clinical results. *J Clin Periodontol* 2002;29:54–61. [PubMed: 11846850]
5. Mascarenhas P, Gapski R, Al-Shammari K, et al. Clinical response of azithromycin as an adjunct to non-surgical periodontal therapy in smokers. *J Periodontol* 2005;76:426–436. [PubMed: 15857078]
6. Jain R, Danziger LH. The macrolide antibiotics: a pharmacokinetic and pharmacodynamic overview. *Curr Pharm Des* 2004;10:3045–3053. [PubMed: 15544496]
7. Pajukanta R, Asikainen S, Saarela M, Alaluusua S, Jousimies-Somer H. In vitro activity of azithromycin compared with that of erythromycin against *Actinobacillus actinomycetemcomitans*. *Antimicrob Agents Chemother* 1992;36:1241–1243. [PubMed: 1329617]
8. Pajukanta R. In vitro susceptibility of *Porphyromonas gingivalis* to azithromycin, a novel macrolide. *Oral Microbiol Immunol* 1993;8:325–326. [PubMed: 8265209]
9. Piccolomini R, Catamo G, Di Bonaventura G. Bacteriostatic and bactericidal in vitro activities of clarithromycin and erythromycin against periodontopathic *Actinobacillus actinomycetemcomitans*. *Antimicrob Agents Chemother* 1998;42:3000–3001. [PubMed: 9797240]
10. Goldstein EJC, Citron DM, Merriam CV, Warren Y, Tyrrell K. Activities of telithromycin compared to those of erythromycin, azithromycin, clarithromycin, roxithromycin and other antimicrobial agents against unusual anaerobes. *Antimicrob Agents Chemother* 1999;43:2801–2805. [PubMed: 10543769]
11. Merriam CV, Citron DM, Tyrrell KL, Warren YA, Goldstein EJ. In vitro activity of azithromycin and nine comparator agents against 296 strains of oral anaerobes and 31 strains of *Eikenella corrodens*. *Int J Antimicrob Agents* 2006;28:244–248. [PubMed: 16875802]

12. Sefton AM, Maskell JP, Beighton D, et al. Azithromycin in the treatment of periodontal disease. Effect on microbial flora. *J Clin Periodontol* 1996;23:998–1003. [PubMed: 8951627]
13. Amsden GW. Advanced-generation macrolides: tissue-directed antibiotics. *Int J Antimicrob Agents* 2001;18(Suppl 1):S11–S15. [PubMed: 11574189]
14. Scaglione F, Demartini G, Dugnani S, Fraschini F. A new model examining intracellular and extracellular activity of amoxicillin, azithromycin, and clarithromycin in infected cells. *Chemotherapy* 1993;39:416–423. [PubMed: 8222870]
15. Chou, C-H. Clarithromycin transport by gingival fibroblasts and epithelial cells. [Thesis]. Columbus, OH: The Ohio State University; 2007. p. 27
16. Amsden GW. Anti-inflammatory effects of macrolides—an underappreciated benefit in the treatment of community-acquired respiratory tract infections and chronic inflammatory pulmonary conditions? *J Antimicrob Chemother* 2005;55:10–21. [PubMed: 15590715]
17. Retsema JA, Bergeron JM, Girard D, Milisen WB, Girard AE. Preferential concentration of azithromycin in an infected mouse thigh model. *J Antimicrob Chemother* 1993;31(Suppl E):5–16. [PubMed: 8396096]
18. Fietta A, Merlini C, Gialdroni Grassi G. Requirements for intracellular accumulation and release of clarithromycin and azithromycin by human phagocytes. *J Chemother* 1997;9:23–31. [PubMed: 9106014]
19. Anderson R, Joone G, van Rensburg CE. An in-vitro evaluation of the cellular uptake and intraphagocytic bioactivity of clarithromycin (A-56268, TE-031), a new macrolide antimicrobial agent. *J Antimicrob Chemother* 1988;22:923–933. [PubMed: 2977384]
20. Gladue RP, Bright GM, Isaacson RE, Newborg MF. In vitro and in vivo uptake of azithromycin (CP-62,993) by phagocytic cells: possible mechanism of delivery and release at sites of infection. *Antimicrob Agents Chemother* 1989;33:277–282. [PubMed: 2543276]
21. McDonald PJ, Pruul H. Phagocyte uptake and transport of azithromycin. *Eur J Clin Microbiol Infect Dis* 1991;10:828–833. [PubMed: 1662626]
22. Ives TJ, Schwab UE, Ward ES, Butts JD, Hall IH. Disposition and functions of clarithromycin in human THP-1 monocytes during stimulated and unstimulated conditions. *Res Commun Mol Pathol Pharmacol* 2001;110:183–208. [PubMed: 12760488]
23. Hall IH, Schwab UE, Ward ES, Butts JD, Wolford ET, Ives TJ. Disposition and intracellular activity of azithromycin in human THP-1 acute monocytes. *Int J Antimicrob Agents* 2002;20:348–360. [PubMed: 12431870]
24. Gladue RP, Snider ME. Intracellular accumulation of azithromycin by cultured human fibroblasts. *Antimicrob Agents Chemother* 1990;34:1056–1060. [PubMed: 2168141]
25. Loe H, Silness J. Periodontal Disease in Pregnancy. I Prevalence and Severity. *Acta Odontol Scand* 1963;21:533–551. [PubMed: 14121956]
26. Silness J, Loe H. Periodontal Disease in Pregnancy. II Correlation between Oral Hygiene and Periodontal Condition. *Acta Odontol Scand* 1964;22:121–135. [PubMed: 14158464]
27. Preshaw PM, Kelly PJ, Heasman PA. Quadratic calibration curves for the Periotron 6000. *J Periodontol Res* 1996;31:441–443. [PubMed: 8884638]
28. Loe H, Theilade E, Jensen SB. Experimental Gingivitis in Man. *J Periodontol* 1965;36:177–187. [PubMed: 14296927]
29. Sastre Torano J, Guchelaar HJ. Quantitative determination of the macrolide antibiotics erythromycin, roxithromycin, azithromycin and clarithromycin in human serum by high-performance liquid chromatography using pre-column derivatization with 9-fluorenylmethyloxycarbonyl chloride and fluorescence detection. *J Chromatogr B Biomed Sci Appl* 1998;720:89–97. [PubMed: 9892071]
30. Blandizzi C, Malizia T, Lupetti A, et al. Periodontal tissue disposition of azithromycin in patients affected by chronic inflammatory periodontal diseases. *J Periodontol* 1999;70:960–966. [PubMed: 10505797]
31. Lavda M, Clausnitzer CE, Walters JD. Distribution of systemic ciprofloxacin and doxycycline to gingiva and gingival crevicular fluid. *J Periodontol* 2004;75:1663–1667. [PubMed: 15732869]
32. Rodvold KA. Clinical pharmacokinetics of clarithromycin. *Clin Pharmacokinet* 1999;37:385–398. [PubMed: 10589373]

33. Zalewska-Kaszburska J, Gorska D. Anti-inflammatory capabilities of macrolides. *Pharmacol Res* 2001;44:451–454. [PubMed: 11735349]
34. Gotfried MH. Macrolides for the treatment of chronic sinusitis, asthma, and COPD. *Chest* 2004;125:52S–60S. [PubMed: 14872001]
35. Darany DG, Beck FM, Walters JD. The relationship of gingival fluid leukocyte elastase activity to gingival fluid flow rate. *J Periodontol* 1992;63:743–747. [PubMed: 1474474]
36. Mirodzhev GK, Ishankulova DM, Boimatova MB, Negmatova FA. Side effects of *Helicobacter pylori* eradication therapy. *Klin Med (Mosk)* 2007;85:47–50. [PubMed: 17682493]

Table 1

Mean Gingival Index values at control and gingivitis sites[§]

Site	Day 0(Baseline)	Day 7	Day 14	Day 21 (After Establishment of Gingivitis)	Day 24 (After Clarithromycin Regimen)
Control	0.0 (0.0)	0.0 (0.0)*	0.0(0.0)*	0.125 (0.25)*	0.125 (0.25)*
Exp.Gingivitis	0.0 (0.25)	1.0 (0.75)*	1.25 (1.5)*	1.75 (0.50)*	1.75 (0.50)*

[§]Data are expressed as median and range of eight subjects. Within columns, values that are statistically significant are denoted by * ($P = 0.008$, Wilcoxon Signed Rank test).

Table 2

Mean Plaque Index values at control and gingivitis sites[§]

Site	Day 0(Baseline)	Day 7	Day 14	Day 21 (After Establishment of Gingivitis)	Day 24 (After Clarithromycin Regimen)
Control	0.0 (0.0)	0.125 (0.25)*	0.0 (0.25)*	0.0 (0.0)*	0.0 (0.25)*
Gingivitis	0.0 (0.50)	1.25 (1.25)*	1.625 (2.25)*	2.0 (1.5)*	1.25 (2.25)*

[§]The data are reported as median and range of eight subjects. Within columns, values that are statistically significant are denoted by * ($P = 0.008$, Wilcoxon Signed Rank test).

Table 3Mean gingival crevicular fluid flow rate at control and gingivitis sites[§]

Site	Day 0(Baseline)	Day 24 (After Establishment of Gingivitis and Administration of Clarithromycin)
Control	0.134 ± 0.020	0.044 ± 0.010 ^{^*}
Exp. Gingivitis	0.152 ± 0.017	0.232 ± 0.023 ^{^#}

[§]The data are expressed as mean flow rate (in μ l per 45 seconds) $\pm$ SEM. Baseline values at control and experimental gingivitis sites were very similar ($P = 0.54$). On day 24, there was a statistically significant difference between control and experimental gingivitis sites ([^] denotes $P < 0.001$). Within rows, significant differences from baseline are denoted (^{*} $P = 0.015$, [#] $P = 0.018$, paired t tests adjusted for multiple comparisons).

Table 4Mean clarithromycin levels in serum, healthy control gingiva and inflamed gingiva[§]

Subject Group	Number of Subjects	Serum Concentration(μ g/ml)	Tissue Concentration at Control Sites(μ g/g)	Tissue Concentration at Gingivitis Sites(μ g/g)
All Subjects	8	0.50 ± 0.08	$2.40 \pm 0.27^*$	$3.00 \pm 0.35^*$
Serum level ≤ 0.4 μ g/ml	4	$0.37 \pm 0.03^\#$	$3.02 \pm 0.29^\#$	$3.71 \pm 0.33^\#$
Serum level > 0.4 μ g/ml	4	0.64 ± 0.12	$1.78 \pm 0.10^*$	$2.29 \pm 0.36^*$

[§]Data are presented as mean $\pm$ SEM. Within rows, * denotes values that were significantly different from serum levels, while # indicates data in which all three pairwise comparisons yielded significant differences ($P < 0.05$, Holm-Sidak test).

Table 5

Ratio of tissue clarithromycin concentration to serum clarithromycin concentration in healthy control gingiva and gingivitis sites[§]

Subject Group	Number of Subjects	Control	Exp. Gingivitis
All Subjects	8	5.66 ± 1.14 [*]	7.00 ± 1.36 [*]
Serum level ≤0.4 µg/ml	4	8.25 ± 1.15 [*]	10.1 ± 1.20 [*]
Serum level >0.4 µg/ml	4	3.07 ± 0.48	3.88 ± 0.84

[§]Data are expressed as mean ± SEM. Within rows, * denotes a significant difference between values ($P \leq 0.02$, paired t test).

* Periopaper, Oraflow Inc, Plainview, NY, USA.

[†] Periotron 6000, Oraflow, Inc, Plainview, NY, USA.

[§] Supelcosil, Sigma-Aldrich, St. Louis, MO, USA.

[¶] United States Pharmacopeia, Rockville, MD, USA.