Gingival Crevicular Fluid Levels of Clindamycin Compared with Its Minimal Inhibitory Concentrations for Periodontal Bacteria

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Clindamycin concentrations in gingival crevicular fluid and in blood were determined over a 7-h period and were related to the minimal inhibitory concentrations of this agent for 340 bacterial strains isolated from diseased periodontal sites. The clindamycin levels after administration of single 300-mg oral doses were measured in gingival crevicular fluids by using an agar diffusion bioassay. Minimal inhibitory concentrations were determined by agar dilution techniques for 30 species of periodontal bacteria. With the exception of Eikenella corrodens and Actinobacillus actinomycetemcomitans, most of the bacteria were inhibited by a concentration of 1.0 μg of clindamycin per ml or less. The peak concentrations in crevicular fluid (2.0 ± 0.3 μg/ml) and in blood (1.9 ± 0.3 μg/ml) were approximately the same. However, crevicular fluid levels of 1.0 μg/ml and above were present for up to 6 h, whereas blood concentrations dropped below 1.0 μg/ml within 2 h after administration. Based on its minimal inhibitory concentrations, clindamycin at crevicular fluid levels of 1.0 μg/ml or above should inhibit most bacteria associated with diseased periodontal sites.

The recognition that destructive periodontal diseases are probably caused by specific bacteria has heightened interest in the use of antimicrobial agents as adjuncts in periodontal therapy. Tetracycline has been the most frequently used antibiotic for treatment of periodontal disease in the United States. Data from antibiotic susceptibility testing (15; C. B. Walker, J. M. Gordon, S. J. McQuilkin, T. A. Niebloom, and S. S. Socransky, J. Periodontol., in press) and pharmacokinetic studies (4; J. M. Gordon, C. B. Walker, J. C. Murphy, J. M. Goodson, and S. S. Socransky, J. Clin. Periodontol., in press) have shown that tetracycline is delivered in sufficient quantities to inhibit most organisms at the site of infection, which in periodontal diseases is the pocket or space between the teeth and the gingiva. However, resistance to this drug is not uncommon (15, 18) and may account for patients who continue to lose alveolar bone despite conventional therapy and the adjunctive use of tetracycline. In some patients, susceptibility data have indicated that penicillin may be as effective as tetracycline (7, 15). However, little is known regarding the penetration of penicillin into the gingival crevicular fluid and its possible degradation by extracellular beta-lactamases. There is currently little information concerning the efficacy of other antibiotics in periodontal therapy. Previous studies have indicated that clindamycin is an effective agent against anaerobic bacterial infections in other parts of the body (6, 12, 13) and, therefore, may be an alternative for use against oral infections when tetracycline and penicillin are contraindicated due to bacterial resistance or adverse reactions. Ideally, before an antimicrobial agent is used, a clinician should know whether the agent is inhibitory for the bacteria involved and whether a therapeutic dosage can be delivered to the site of the infection. In this investigation, we determined the susceptibility of periodontal bacteria to clindamycin and measured the gingival crevicular fluid levels of this drug with a modified agar diffusion assay. We found that most bacteria in the periodontal pocket were susceptible to clindamycin at levels readily achieved in crevicular fluid.

MATERIALS AND METHODS

Bacterial strains. We tested 340 characterized strains, representing 30 species of bacteria frequently isolated from human periodontal pockets. As a reference strain, we used the nonoral strain Bacteroides fragilis VP1 8708AP, whose minimal inhibitory concentrations (MICs) and growth characteristics were described previously (C. B. Walker, Ph.D. thesis, Virginia Polytechnic Institute and State University, Blacksburg, 1977).

Cultivation. Before the study, stock cultures were preserved in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) that was supplemented with 0.5% di-
methyl sulfoxide and frozen in liquid N₂. During the study, all strains except *Bacteroides gingivalis* (formerly *Bacteroides asaccharolyticus* [2]) and *B. melaninogenicus* subspecies were maintained on Trypticase soy blood agar (BBL Microbiology Systems, Cockeysville, Md.) and transferred weekly. Strains of *B. gingivalis* and *B. melaninogenicus* subspecies were maintained on Trypticase soy blood agar supplemented with 5 µg of hemin (equine III; Sigma Chemical Co., St. Louis, Mo.) per ml and 0.5 µg of menadione (Sigma) per ml and transferred weekly. All strains were incubated at 35°C in an anaerobic chamber containing an atmosphere of 80% N₂, 10% H₂, and 10% CO₂.

**Broth media.** The following broth media were used: *Mycoplasma* broth base (BBL) supplemented with 0.3% glucose (MBM); MBM supplemented with 0.1% sodium formate and 0.15% sodium fumarate; MBM supplemented with 0.1% KNO₃ and CS4 broth (17).

**Broth cultures.** Broth inocula were prepared by transferring three to five colonies from Trypticase soy broth agar to a broth medium. *Fusobacterium*, *Capnocytophaga*, *Streptococcus*, *Selenomonas*, and *Peptostreptococcus* strains were inoculated into MBM. *Actinobacillus actinomycetemcomitans* was inoculated into MBM to which filter-sterilized NaHCO₃ was added at the time of inoculation to give a final concentration of 1 mg/mL. *B. gingivalis*, *B. melaninogenicus*, and *Bacteroides oralis* were inoculated into MBM supplemented with 0.5 µg of menadione per ml. *Wolinella recta* (anaerobic Vibrio), *Campylobacter concisus* (oral *Campylobacter*), and *Bacteroides gracilis* ("corroding" *Bacteroides*) (S. J. Badger and A. C. R. Tanner, submitted for publication) were inoculated into MBM supplemented with 0.1% sodium formate and 0.15% sodium fumarate. *Eikenella corrodens* was inoculated into MBM supplemented with 0.1% KNO₃. CS4 broth was used for *Actinomyces*, *Propionibacterium acnes*, and *Lactobacillus* strains. All broth cultures were inoculated by using a VPI anaerobic culture system (Bellco Glass, Inc., Vineland, N.J.) and an O₂-free gas mixture containing 80% N₂, 10% H₂, and 10% CO₂. Cultures were incubated at 35°C for 24 to 48 h.

**MICs.** The MICs of clindamycin were determined by using clindamycin hydrochloride susceptibility powder (The Upjohn Co., Kalamazoo, Mich.) and an agar dilution technique, as previously described (16). The MIC was defined as the lowest concentration of antibiotic that gave no growth.

**Agar diffusion assay method.** The assay used was a modification of the assay described by Sabath and Toftegaard (8). The modifications described below were necessary to increase the sensitivity of the assay approximately 50-fold, since less than 0.5 µl of crevicular fluid could be collected readily from each gingival crevice site. The assay organism, *Clostridium perfringens* ATCC 13124, was maintained by weekly transfers in Todd-Hewitt broth. A seed culture was prepared by transferring 0.8 ml of the stock culture to 9.0 ml of Todd-Hewitt broth and incubating this preparation anaerobically overnight; 0.4 ml of the overnight culture was added to 9.0 ml of Todd-Hewitt broth, and the resulting culture was incubated anaerobically for approximately 2.5 h and then adjusted to an optical density at 550 nm of 0.15.

The assay medium was prepared by adding 0.5 g of agar (Ionagar; Colab Laboratories, Glenwood, Ill.) to 100 ml of brain heart infusion broth (BBL). The brain heart infusion agar medium was adjusted to pH 8.0, autoclaved, and cooled to 46°C. Then, 8 ml of defibrinated whole sheep blood (BBL) and 2.0 ml of the standardized seed culture were added to the medium and 17-ml volumes were poured into large petri plates (150 by 15 mm; VanLab, Boston, Mass.) and allowed to solidify.

Sample strips of gingival fluid and two series of standards were placed onto the assay plates, and the plates were incubated anaerobically at 35°C for 4 to 5 h. Because the zones of inhibition of hemolysis were often elliptical, diameters were measured along both the long axes and the short axes to the nearest 0.1 mm, and the averages were recorded. Blood samples were assayed by the same procedure, except that standards were prepared in defibrinated sheep blood (BBL). Preliminary trials demonstrated that sheep blood was a suitable substitute for pooled human blood and yielded approximately equivalent inhibition zone diameters.

**Preparation of standards.** A stock solution (1.0 mg/ml) was prepared by dissolving clindamycin hydrochloride in distilled water. Lower concentrations (0.125 through 4.0 µg/ml) were made by dilution with phosphate-buffered 7.0% bovine serum albumin, as previously described (4); 0.5-µl amounts of standard solutions were placed onto filter paper strips (Peripaper; Harco Electronics, Winnipeg, Ontario, Canada) with a 1.0-µl syringe. Standard strips could be stored at −20°C for up to 6 weeks without loss of activity.

**Standard curves.** Standard curves were fitted by linear regression, using the method of Colquhoun (1). Since the volumes of the gingival fluid samples varied, zone diameters were converted to absolute amounts of clindamycin per strip, and the concentration in each gingival fluid sample was calculated.

**Human subjects.** Three volunteers were given single oral 300-mg doses of clindamycin hydrochloride (Cleocin; The Upjohn Co.). Gingival fluids and blood were sampled at 30-min intervals for the first 2 h and then at 1-h intervals for an additional 5 h. Blood was collected by finger puncture. Gingival crevicular fluid was collected from four sites in each subject, and the volume from each site was determined by using a gingival fluid meter (Harco Electronics), as previously described (4). All volunteers had minimal or no clinically evident inflammation and had not received anti-biotic therapy for at least 3 months before the study.

**RESULTS**

**MICs.** The MICs of clindamycin for 340 strains of periodontal bacteria are summarized in Table 1. With the exception of *E. corrodens* and *A. actinomycetemcomitans*, most bacteria were inhibited by a concentration of 1 µg of clindamycin per ml or less.

**Precision and accuracy of the assay.** The precision of the assay for clindamycin was deter-
TABLE 1. Susceptibilities of periodontal bacteria to clindamycin

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC of clindamycin (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Actinomyces israelii (12)*</td>
<td>≤0.06-2</td>
</tr>
<tr>
<td>Actinomyces naeslundii (13)</td>
<td>≤0.06-8</td>
</tr>
<tr>
<td>Actinomyces odontolyticus (4)</td>
<td>≤0.06-0.25</td>
</tr>
<tr>
<td>Actinomyces viscosus (12)</td>
<td>≤0.06-1</td>
</tr>
<tr>
<td>Actinobacillus actinomycetemcomitans (23)</td>
<td>≤0.06 - &gt;32</td>
</tr>
<tr>
<td>Bacteroides gingivalis (15)</td>
<td>≤0.06</td>
</tr>
<tr>
<td>Bacteroides melaninogenicus subsp. intermedius (23)</td>
<td>≤0.06-0.5</td>
</tr>
<tr>
<td>B. melaninogenicus subsp. melaninogenicus (16)</td>
<td>≤0.06-1</td>
</tr>
<tr>
<td>Bacteroides gracilis (15)</td>
<td>≤0.06</td>
</tr>
<tr>
<td>Other Bacteroides (10)</td>
<td>≤0.06</td>
</tr>
<tr>
<td>Campylobacter concisus (6)</td>
<td>0.125-2</td>
</tr>
<tr>
<td>Capnocytophaga species (24)</td>
<td>≤0.06</td>
</tr>
<tr>
<td>Eikenella corrodens (17)</td>
<td>4-&gt;32</td>
</tr>
<tr>
<td>Eubacterium species (6)</td>
<td>≤0.06-0.125</td>
</tr>
<tr>
<td>Fusobacterium nucleatum (41)</td>
<td>≤0.06-0.5</td>
</tr>
<tr>
<td>Fusobacterium goridisfermens (9)</td>
<td>≤0.06-0.25</td>
</tr>
<tr>
<td>Other Fusobacterium (9)</td>
<td>≤0.06-0.25</td>
</tr>
<tr>
<td>Lactobacillus species (6)</td>
<td>≤0.06-0.25</td>
</tr>
<tr>
<td>Selenomonas putitigera (9)</td>
<td>≤0.06-1</td>
</tr>
<tr>
<td>Streptococcus (34)</td>
<td>≤0.06-1</td>
</tr>
<tr>
<td>Peptostreptococcus (15)</td>
<td>≤0.06-0.25</td>
</tr>
<tr>
<td>Propionibacterium acnes (4)</td>
<td>≤0.06-0.125</td>
</tr>
<tr>
<td>Wolinella recta (17)</td>
<td>≤0.06-1</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are numbers of strains tested.

mined by measuring zone diameters produced by replicate standards. Standards were prepared by placing 0.5-μl portions of known concentrations of clindamycin onto filter paper strips. Six strips of each concentration were assayed on the same plate. The resulting regression line and the prediction interval (5) are shown in Fig. 1. The confidence interval, which was narrower than the prediction interval, ranged from ±5% for the highest concentration of clindamycin to ±16% for the lowest concentration.

The accuracy of the assay was determined by randomly selecting duplicate standards from the replicate standards described above. The duplicate standards were used as known concentrations, and the remaining replicates of each concentration were unknown concentrations. A regression line was fitted to the duplicate standards, and the values of the unknown concentrations were predicted. The absolute differences between the predicted values and the actual concentrations on the strips were determined and expressed as percentages of the known concentrations. The error was 14.9 ± 9.04% (mean ± standard deviation).

Gingival crevicular fluid levels. Figure 2 shows the mean crevicular fluid levels and the mean blood levels for the three subjects. Clindamycin reached maximum concentrations in the blood (1.9 ± 0.3 μg/ml) in approximately 30 min and in the crevicular fluids (2.0 ± 0.3 μg/ml) in about 90 min after administration. Although the peak concentration in the crevicular fluid was approximately the same as the peak blood level in each volunteer, clindamycin persisted at higher levels in the crevicular fluids than in the blood. Crevicular fluid levels were more than 1 μg/ml for 6 h after the single oral dose and were significantly higher (P < 0.001) than blood levels after the second hour.

DISCUSSION

Because of the small volume of crevicular fluid that can be collected conveniently from a gingival site at one time, it was necessary to optimize the sensitivity of an agar diffusion assay (8) in order to measure clindamycin concentrations in crevicular fluids. The most important parameters that influenced the sensitivity of the assay were (i) the composition of the agar medium, (ii) the turbidity of the assay inoculum, and (iii) the depth of the assay medium. This assay was as sensitive and as reproducible as a similar assay.
**FIG. 1.** Precision of the clindamycin assay. The responses of six replicates of each of six concentrations over the useful range were determined. The regression line (solid line) and the 95% prediction interval (dashed lines) are shown. The numbers on the figure represent the numbers of coincident points.

**FIG. 2.** Clindamycin concentrations in crevicular fluid (dashed line) and blood (solid line) after oral administration of 300 mg of clindamycin hydrochloride. The means ± standard deviations are shown for 12 samples of crevicular fluid and 3 samples of blood for each sampling period.

previously described for the quantitation of tetracycline in crevicular fluids (4). Clindamycin reached approximately the same peak concentrations in crevicular fluid and blood, although the peak concentration in crevicular fluid occurred about 1 h later than the peak concentration in blood. However, after the peak concentration was reached, clindamycin remained at higher concentrations in crevicular fluid than in blood, which indicated that higher
bacteriostatic levels of this drug may be maintained in crevicular fluid after recommended dosages.

A comparison of the MICs of clindamycin required to inhibit most periodontal bacteria indicated that this agent should be effective as an adjunct in periodontal therapy. Only strains of A. actinomyctetemcomitans and E. corrodens were not inhibited effectively. However, we found that our strains of A. actinomyctetemcomitans were more susceptible to clindamycin than the strains used by Slots et al. (10). This difference in susceptibility may have been caused by differences in the isolates tested or by differences in the susceptibility media used or both. All of the E. corrodens isolates tested were relatively resistant to clindamycin. This seems to be a characteristic of this species since several media for the selective recovery of this organism depend on clindamycin as the selective agent (C. B. Walker, A. C. R. Tanner, C. Smith, and S. Sooransky, J. Dent. Res. 57A:315, abstr. 961, 1978; A. M. Slee and J. M. Tanzer, J. Dent. Res. 57A:314, abstr. 960, 1978). Both A. actinomyctetemcomitans and E. corrodens have been implicated or correlated with certain forms of destructive periodontal disease (14). Organisms thought to be periodontopathic (3, 9, 11) that were susceptible to clindamycin included B. ginvialis, B. melaninogenicus subspecies, other Bacteroides species, Fusobacterium nucleatum, W. recta, and Selenomonas sputigena. With the possible exception of B. ginvialis, all of these taxa include strains which are relatively resistant to tetracycline (15). In addition, strains of B. melaninogenicus subsp. intermedius, B. oralis, and S. sputigena have been isolated which were not inhibited in vitro by either tetracycline or penicillin and appear to contain an active beta-lactamase located in the interplasmic space (Walker, unpublished data). These strains were included in this study and were inhibited by <1.0 µg of clindamycin per ml.

In conclusion, representative strains of most of the bacteria which have been cultivated from periodontal pockets were inhibited in vitro by clindamycin concentrations that were achieved in crevicular fluid. We believe that it may be possible to use clindamycin as an adjunct to conventional periodontal therapy when tetracycline and penicillin are contraindicated. However, in view of the intestinal disorders associated with clindamycin usage, clinical trials are needed to evaluate fully the risk-to-benefit potential of this drug in periodontal therapy.

ACKNOWLEDGMENTS

This investigation was supported by a grant from The Upjohn Co. and by Public Health Service grant 5T32-DE-07010 and Public Health Service Periodontal Disease Clinical Research Center Grant DE-04881 from the National Institute of Dental Research.

LITERATURE CITED