Bacteriostatic and Bactericidal In Vitro Activities of Clarithromycin and Erythromycin against Periodontopathic
Actinobacillus actinomycetemcomitans

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The susceptibilities of 87 periodontitis-associated strains of Actinobacillus actinomycetemcomitans to clarithromycin and erythromycin were determined by standard methodology recommended for Haemophilus influenzae. For clarithromycin the MIC at which 90% of the isolates were inhibited was ≤2.0 μg/ml and the minimal bactericidal concentration at which 90% of the strains were killed was ≤4.0 μg/ml, suggesting that it would be a candidate for therapeutic trials in patients with periodontitis.

Actinobacillus actinomycetemcomitans has been strongly implicated as the agent responsible for juvenile (3) and adult (19) progressive periodontitis. Given that periodontal patients with a positive finding for A. actinomycetemcomitans often fail to respond adequately to mechanical therapy only (8), many antibiotics have been studied for treatment of A. actinomycetemcomitans-associated periodontal diseases (1, 2, 6, 18); however, tibiotics have been studied for treatment of patients who respond adequately to mechanical therapy only (8), many antibiotics have been studied for treatment of A. actinomycetemcomitans.-associated periodontitis.

For this study 87 periodontitis-associated strains of A. actinomycetemcomitans were directly isolated during the year 1997 by criteria described previously (13), and three strains of A. actinomycetemcomitans were obtained from commercial sources (ATCC 29522 and ATCC 29523 from the American Type Culture Collection, Rockville, Md., and NCTC 9710 from the National Collection of Type Cultures, London, United Kingdom). Pure cultures of A. actinomycetemcomitans were obtained in Trypticase soy agar and horse serum containing 75 μg of bacitracin and 5 μg of vancomycin per ml (17). These pure cultures were identified to the genus, species, and serotype levels by criteria previously reported (14). Haemophilus influenzae ATCC 49247 and Staphylococcus aureus ATCC 29213 were employed as controls. The MIC was determined by the agar dilution method on Mueller-Hinton Haemophilus test medium (5) on the basis of guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (11).

Freshly prepared solutions of twofold dilutions of clarithromycin and erythromycin were incorporated into the above-described medium to yield final concentrations ranging from 64 to 0.125 μg/ml. The final inoculum for each strain contained 106 CFU per spot. The MIC was recorded as the lowest macrolide concentration totally inhibiting visible bacterial growth on the agar surface after 48 h of incubation at 37°C in 5% CO2. The breakpoints recommended by the NCCLS for defining susceptible, intermediate, and resistant H. influenzae strains were ≤0.5, 1 to 4, and ≥8 μg/ml, respectively, for erythromycin and ≤2, 4, and ≥8 μg/ml, respectively, for clarithromycin. No standardized breakpoint is currently available for the action of these compounds against A. actinomycetemcomitans, a bacterium closely related to H. influenzae. Therefore, in this study the breakpoints for susceptibility and resistance to the two macrolides employed were those recommended in NCCLS methods for susceptibility testing of H. influenzae.

To determine the minimum bactericidal concentration (MBC) the broth MIC was determined with Mueller-Hinton broth Haemophilus test medium. This test was done according to the NCCLS guidelines by broth microdilution procedures with a final inoculum of 5 × 108 CFU/ml. The MIC was defined as the lowest macrolide concentration that inhibited visible growth in broth after incubation for 48 h at 37°C in a 5% CO2 incubator. The MBC was determined by subculturing 0.01 ml of broth from a well without visible growth to a Mueller-Hinton Haemophilus test medium agar plate. The MBC was defined as the lowest concentration yielding no more than 0.1% survival of the initial inoculum (99.9% killing) after incubation of the subcultures for 48 h at 37°C in 5% CO2.

In vitro activities of clarithromycin and erythromycin against the 87 clinical strains of A. actinomycetemcomitans are given in Table 1. With the exception of two clarithromycin-resistant (MIC = 8.0 μg/ml) and three clarithromycin-intermediate (MIC = 4.0 μg/ml) serotype b subpopulation strains of A. actinomycetemcomitans, all clinically isolated A. actinomycetemcomitans strains, plus the three reference strains, were inhibited by clarithromycin at an MIC of 2.0 μg/ml or less; 100% of these 82 clinically isolated strains were inhibited by erythromycin at an MIC of 16.0 μg/ml. For serotype a and...
serotype c subpopulations of A. actinomycetemcomitans, clarithromycin exhibited MICS at which 50 and 90% of the isolates were inhibited (MIC_{50} and MIC_{90}) of 0.25 and 1.0 μg/ml, respectively. For 34 serotype b subpopulations of A. actinomycetemcomitans, the MIC_{50} and MIC_{90} of clarithromycin were 1.0 and 4.0 μg/ml, respectively. The clarithromycin and erythromycin MICS obtained by the conventional agar dilution and broth microdilution methods were similar. The MBCs of clarithromycin were always either the same as the MIC or, at most, twofold higher than the corresponding MIC result. Erythromycin MBCs for 53 of the 87 A. actinomycetemcomitans strains of clinical origin were three- to fourfold higher than the corresponding MIC, and those for the other strains were twofold higher than the corresponding MIC.

It is well known that the proper use of antibiotics in periodontal chemotherapy is based on a knowledge of the concentrations required to inhibit growth of the bacterium involved and on a knowledge of how these MICS compare with the levels of the particular antibiotics attainable at the oral site of infection. Thus, our data for the MICS and MBCs of clarithromycin for A. actinomycetemcomitans seem to warrant in vivo studies and/or therapeutic trials of this causative agent in some forms of severe periodontitis. Clarithromycin is well tolerated at a dose of 500 mg given once daily and may have a lower incidence of side effects, better bioavailability, and a more favorable pharmacokinetic profile than erythromycin. Clarithromycin is also characterized by the ability to achieve considerable concentrations in serum and in saliva: 2.3 and 1.1 μg/ml, respectively, were measured following a 500-mg oral dose given once a day (7). Moreover, in vitro and ex vivo studies have shown that clarithromycin achieves high intracellular and extracellular concentrations (7, 15, 16), which is an outstanding feature of this macrolide and may contribute to its excellent efficacy against various intracellular and extracellular bacteria, such as A. actinomycetemcomitans (9).

In conclusion, the results of this study indicate that clarithromycin is highly effective in vitro against A. actinomycetemcomitans; 94% of the strains were inhibited at a concentration of ≤2.0 μg/ml, a susceptibility value which can be tentatively assumed to be the breakpoint for susceptibility of A. actinomycetemcomitans to clarithromycin. Clinical studies to investigate this potential therapeutic application are required.

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REFERENCES

TABLE 1. In vitro activities of clarithromycin and erythromycin against 87 recent clinical isolates of A. actinomycetemcomitansa

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (μg/ml)</th>
<th>MBC (μg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>Range 50%</td>
<td>90%</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>0.12–8.0</td>
<td>0.5 2.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.5–32.0</td>
<td>2.0 16.0</td>
</tr>
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a Serotype a, 26 isolates; serotype b, 34 isolates; serotype c, 27 isolates.