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# In Vitro Activity of Azithromycin Compared with That of Erythromycin against *Actinobacillus actinomycetemcomitans*

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**The in vitro susceptibility of *Actinobacillus actinomycetemcomitans* to azithromycin, a new macrolide antibiotic of a new class known as azalides, was compared with that of erythromycin by the agar dilution method on Mueller-Hinton *Haemophilus* test medium. Eighty-two *A. actinomycetemcomitans* strains, 79 recent clinical isolates obtained from 40 periodontally healthy or diseased subjects, and 3 type strains were included in the study. Erythromycin showed poor in vitro activity against *A. actinomycetemcomitans*. Azithromycin, however, was highly effective against *A. actinomycetemcomitans*: all strains were inhibited at 2.0 μg/ml. Azithromycin exhibited the best in vitro activity against the serotype *a* subpopulation of *A. actinomycetemcomitans*: 100% of the strains were inhibited at 1.0 μg/ml. The lowest MICs were, however, recorded by serotype *b* strains. Since azithromycin has favorable pharmacokinetic properties, including excellent distribution into tissues, it could be expected to pass into gingival crevicular fluid at levels sufficient to inhibit *A. actinomycetemcomitans* in vivo. Therefore, it is a good candidate for future clinical trials in *A. actinomycetemcomitans*-associated periodontitis.**

*Actinobacillus actinomycetemcomitans* is a capnophilic gram-negative coccobacillus which has been associated with localized juvenile periodontitis and with some cases of refractory and rapidly progressing periodontitis (33). The distribution of *A. actinomycetemcomitans* serotypes in periodontally healthy and diseased subjects has lately been reported by Asikainen et al. (3), who demonstrated that serotype *b* was dominant in the subjects with periodontal disease and that serotype *c* was the most common serotype in the healthy subjects. Patients with a positive finding for *A. actinomycetemcomitans* often fail to respond adequately to mechanical therapy only (21). Many antibiotics have been administered in the treatment of *A. actinomycetemcomitans*-associated periodontal diseases. Despite the good in vitro susceptibility of *A. actinomycetemcomitans* to tetracyclines (4, 30), the clinical response has been variable (2, 27, 31). The favorable clinical results obtained with metronidazole are thought to be due to the better in vitro activity of the hydroxy metabolite of metronidazole against *A. actinomycetemcomitans* and the possible synergistic effects of these compounds in vivo (16). However, in some patients metronidazole alone may not be effective enough for the eradication of *A. actinomycetemcomitans* from the oral cavity (20, 25). On the other hand, the production of β-lactamase by other bacterial species in periodontal pockets may compromise the use of β-lactamase-susceptible penicillins (32). Furthermore, the old macrolide antibiotic erythromycin penetrates insufficiently into gingival crevicular fluid (24). Thus, it is necessary to screen for alternative antimicrobial agents that would be active against *A. actinomycetemcomitans* to find potential candidates for therapeutical trials.

During recent years, an extensive development has occurred within the field of macrolides (17, 18). Azithromycin (9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A) is a new macrolide antibiotic, the first of a novel subclass referred to as azalides (9). This agent has been shown to have favorable pharmacokinetic properties, achieving rapid and sustained high concentrations in tissue with oral dosing (11, 28). Azithromycin has already been shown to have in vitro activity against a wide range of pathogens (1, 26) and to be more effective than other macrolide antibiotics against many common pathogens (8). Azithromycin has been shown to be four to eight times more active than erythromycin and roxithromycin against *Haemophilus influenzae* (12), which is closely related to *A. actinomycetemcomitans*. In clinical studies azithromycin has been well tolerated and has had no marked or consistent effect on laboratory safety parameters (13). These properties prompted us to study whether azithromycin is more effective than erythromycin in vitro against *A. actinomycetemcomitans*, the key organism associated with the juvenile and refractory forms of periodontitis.

## MATERIALS AND METHODS

The in vitro study of MICs of azithromycin and erythromycin for *A. actinomycetemcomitans* included 82 *A. actinomycetemcomitans* strains, 79 recent clinical isolates (serotype *a*, 20 isolates; serotype *b*, 32 isolates; serotype *c*, 16 isolates; nontypeable, 11 isolates) cultured on different occasions from 40 subjects, and three type strains (ATCC 29523 [serotype *a*], ATCC 43718 [Y4; serotype *b*], and ATCC 33384 [serotype *c*]). *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were included as aerobic controls. Also, six closely related strains of *Haemophilus* species (three *H. influenzae*, one *H. parainfluenzae*, and two *H. aphrophilus* strains) were included in this study. The *A. actinomycetemcomitans* strains were isolated on selective medium that consisted of tryptic soy agar, serum, bacitracin (75 μg/ml), and vancomycin (5 μg/ml) (29). The identification of the strains was based on Gram staining; nitrate reduction; production of catalase, urease, and indole; growth on MacConkey agar; and fermentation reactions to eight carbohydrates (glucose, sucrose, lactose, maltose, mannitol, galactose, xylose, and fructose) supplemented by the profiles of preformed enzymes (API ZYM system; Bio

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TABLE 1. Comparison of the in vitro activities of azithromycin and erythromycin against *A. actinomycetemcomitans*[a]

| Antimicrobial agent | Cumulative % of strains for which the MIC (μg/ml) was: | | | | | |
|---|---|---|---|---|---|---|
| | 0.25 | 0.5 | 1.0 | 2.0 | 4.0 | 8.0 |
| Azithromycin | 8 | 23 | 80 | 100 | | |
| Erythromycin | | 5 | 8 | 11 | 56 | 100 |

[a] A total of 79 strains were tested for each antimicrobial agent.

Mèrieux SA, La Balme les Grottes, France). The isolates were stored in 20% skim milk at −70°C until they were tested. The MICs of azithromycin (kindly supplied by Pfizer, Helsinki, Finland) and erythromycin base (kindly supplied by Orion, Espoo, Finland) were determined by the agar dilution method on Mueller-Hinton *Haemophilus* test medium (14) on the basis of the latest guidelines of the National Committee for Clinical Laboratory Standards (22). The final inoculum contained $10^4$ CFU per spot and was delivered by using a multipoint inoculator. Following incubation at 35°C in 5% $CO_2$ for 48 h, MICs were interpreted. The effect of the incubation atmosphere on the pH of the test medium was tested by incubating 12 plates that contained different concentrations of azithromycin (0.0, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 μg/ml) in an aerobic atmosphere, in a 5% $CO_2$ atmosphere, and in an anaerobic atmosphere (mixed gas made up of 10% $H_2$, 10% $CO_2$, and 80% $N_2$) obtained by the evacuation replacement method. After incubation, a piece of agar (diameter, 0.5 cm) was taken with a scalpel and was put into a tube containing 2 ml of distilled water, and the pH was immediately recorded.

## RESULTS

The comparative in vitro activities of azithromycin and erythromycin against *A. actinomycetemcomitans* are given in Table 1. Erythromycin exhibited poor in vitro activity against *A. actinomycetemcomitans* and the six closely related *Haemophilus* spp.; the MIC range was 0.5 to 8.0 μg/ml. Erythromycin MICs for the ATCC 29523, ATCC 43718, and ATCC 33384 type strains were 4.0, 8.0, and 8.0 μg/ml, respectively. The corresponding MICs for the aerobic type strains *S. aureus* and *E. coli* were 0.5 and >16.0 μg/ml. All *A. actinomycetemcomitans* strains, including the *A. actinomycetemcomitans* reference strains and *Haemophilus* spp., were inhibited by azithromycin at an MIC of 2.0 μg/ml or less. The azithromycin breakpoint for susceptibility with *Haemophilus* spp. is ≤4.0 μg/ml (5). As indicated by the data in Table 2, azithromycin exhibited the best in vitro activity against the serotype *a* subpopulation of *A. actinomycetemcomitans*; 100% of the strains were inhibited at a concentration of 1.0 μg/ml. However, the lowest MICs were obtained for serotype *b* isolates. Azithromycin MICs for the *S. aureus* and *E. coli* control strains were 2.0 and >4.0 μg/ml, respectively. The minimal and maximal pH values in the different incubation atmospheres were 7.12 and 7.29 (mean, 7.21) for aerobic incubation, 7.10 and 7.34 (mean, 7.20) for 5% $CO_2$ incubation, and 6.45 and 6.68 (mean, 6.56) for anaerobic incubation, respectively. Thus, the 5% $CO_2$ atmosphere had no marked effect on the pH of the test medium when it was compared with the effect of aerobic incubation, whereas the anaerobic incubation lowered the pH of the test medium.

TABLE 2. Susceptibility of *A. actinomycetemcomitans* serotypes to azithromycin

| *A. actinomycetemcomitans* serotype (no. of strains)[a] | No. of strains inhibited at MICs (μg/ml) of: | | | |
|---|---|---|---|---|
| | 0.25 | 0.5 | 1.0 | 2.0 |
| *a* (20) | | 4 | 16 | |
| *b* (32) | 6 | 2 | 11 | 13 |
| *c* (16) | | 3 | 11 | 2 |
| Nontypeable (11) | | 3 | 7 | 1 |

[a] A total of 79 clinical isolates were tested.

## DISCUSSION

The results of the present study indicate that azithromycin is highly effective in vitro against *A. actinomycetemcomitans*; all strains tested in this study were susceptible to azithromycin. In this study, the range of MICs for *A. actinomycetemcomitans* was 0.25 to 2.0 μg/ml and, thus, was 1 dilution step higher than that found in the study of Kitzis et al. (19), who obtained a range of MICs of 0.03 to 1.0 μg/ml for three *A. actinomycetemcomitans* strains after short aerobic (the type of incubation was not reported in the study of Kitzis et al.) incubation. However, from our experience, *A. actinomycetemcomitans* does not grow properly when it is incubated aerobically. It is known that the antimicrobial activities of azithromycin and other macrolides are markedly influenced by the pH of the test medium. In alkaline media, the macrolides are very active, but the activity is reduced 50- to 200-fold when testing is done under acidic conditions (7). In the present study, we showed that anaerobic incubation lowers the pH of the test medium. The MIC results presented here are those obtained in an atmosphere containing 5% $CO_2$.

Our results confirm the previous findings of the poor in vitro activity of erythromycin, a macrolide antibiotic with a 14-membered lactone ring, against *A. actinomycetemcomitans* (4). Erythromycin appears to have very limited application in periodontics, because this drug penetrates into gingival crevicular fluid at levels that are insufficient to inhibit most periodontal organisms (24). Erythromycin exists at low levels in blood, is unstable under the acidic conditions of the stomach, and may cause mild to severe gastrointestinal side effects following oral administration.

Azithromycin is an antimicrobial agent of a new class known as azalides (9) and contains a nitrogen atom in the macrolide aglycone ring. In vitro, it shows greater activity than does erythromycin against gram-negative bacteria including *H. influenzae*, *Neisseria gonorrhoeae*, and members of the family *Enterobacteriaceae* (1, 7, 8, 26). Bactericidal activity is seen for certain streptococci and *H. influenzae* (23). Azithromycin has improved pharmacokinetic properties, including greater stability than that achieved with erythromycin in the presence of acids, excellent distribution into tissues (11, 28), and a long elimination half-life, suggesting that less frequent dosing may be possible. The good penetration of azithromycin into tissues is thought to depend on its ability to concentrate to a high degree within phagocytes and to be transported by chemotactic mechanisms (28). Approximately 37% of a single oral dose of 500 mg of azithromycin has been shown to be bioavailable and to produce a peak concentration in serum of 0.4 μg/ml. The concentrations of azithromycin in tissue are, however, 10 to 100 times higher than those in serum, and they persist after the concentrations in serum have declined (10). In the present study, the activity range of azithromycin to *A. actinomycetemcomitans* was 0.25 to 2.0 μg/ml. Thus, it is theoretically possible that this drug would also pass into the

gingival crevicular fluid at levels sufficiently high to inhibit *A. actinomycetemcomitans* in vivo. As a macrolide, azithromycin is also resistant to β-lactamases, if these were present in periodontal pockets as a product of accompanying organisms in the subgingival flora.

In conclusion, the results of the present study indicate that azithromycin is highly effective in vitro against *A. actinomycetemcomitans*; all 79 strains were inhibited at a concentration of 2.0 μg/ml. Thus, this drug might be a good candidate for future clinical therapy trials of *A. actinomycetemcomitans*-associated periodontitis. In addition, of the other new macrolides, clarithromycin and its 14-hydroxy metabolite have recently been reported to have good in vitro activity against *H. influenzae* (6, 15). As more information about the concentrations of this new drug in tissue and body fluid becomes available, in vitro studies may also be warranted to clarify the efficacy of clarithromycin and its 14-hydroxy metabolite against *A. actinomycetemcomitans*.

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