Comparative Activities of Clarithromycin, Erythromycin, and Azithromycin against Penicillin-Susceptible and Penicillin-Resistant Pneumococci

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Received 1 April 1996/Returned for modification 28 May 1996/Accepted 10 June 1996

Activities of clarithromycin, erythromycin, and azithromycin against 120 pneumococci from the United States were tested by agar dilution MIC. All three compounds yielded MICs at which 90% of the isolates were inhibited (MIC90s) of ≤0.125 µg/ml against penicillin-susceptible and -intermediate strains, but MIC90s against resistant strains were >128.0 µg/ml. All erythromycin-resistant strains were also resistant to clarithromycin and azithromycin. Clarithromycin yielded MICs which were generally one or two dilutions lower than those of the other two compounds for all strains. The respective bacteriostatic and bactericidal values (micrograms per milliliter) for two susceptible, two intermediate, and two resistant strains were 0.004 to 0.03 and 0.016 to 0.03 (0.004 to 0.03/0.016 0.03) (clarithromycin), 0.008 to 0.06/0.016 to 0.125 (erythromycin), and 0.016 to 0.06/0.03 to 0.125 (azithromycin); clarithromycin yielded the lowest values. All compounds were uniformly bactericidal after 24 h only; erythromycin was bactericidal at eight times the MIC, and azithromycin and clarithromycin were both bactericidal at two times the MIC. The relevance of these in vitro differences requires clarification by clinical trials.

The worldwide incidence of infections caused by pneumococci resistant to penicillin G and other antimicrobial agents has increased at an alarming rate during the past 2 decades and in particular during the past 5 years. The main foci of penicillin-resistant pneumococci are currently South Africa, Spain, Eastern Europe, and Korea. However, wherever susceptibility testing is performed by appropriate methods resistant strains are almost universally found (1). The spread of penicillin-resistant clones from country to country and from continent to continent demonstrates the capability of these strains to spread rapidly throughout the world (8, 11). In the United States, recent surveys have shown an increase in resistance to penicillin from <5% before 1989 (including <0.02% of isolates with MICs of ≥2.0 µg/ml) to 6.6% in 1991 to 1992 (including 1.3% of isolates with MICs of ≥2.0 µg/ml) (4).

The distribution of penicillin-resistant strains is highly variable in the United States. Block and coworkers have recently reported a 28% incidence rate of penicillin-resistant pneumococci from middle ear fluid of children with acute otitis media in Kentucky, and a 29% incidence level of these strains in nasopharyngeal cultures from children with otitis media in Tennessee (3).

Pneumococcal strains with intermediate and especially full resistance to penicillin G are often resistant to erythromycin. In the United States Breiman and coworkers in 1991 to 1992 demonstrated erythromycin resistance rates of 3.7% and 2.2% in patients 1 to 2 and ≥4 years of age, respectively (4). In Europe, erythromycin resistance rates are generally higher. For example, 27.5% of all pneumococci studied in France during 1992 (63% of penicillin-resistant strains) were erythromycin resistant (6). Preliminary evidence shows that clarithromycin MICs for pneumococci are generally one or two dilutions lower than those of other macrolides (2, 10, 13); however, pneumococci with erythromycin MICs of ≥64.0 µg/ml are resistant to all macrolides (9). There is an urgent need for compounds active against these resistant pneumococci (5).

In order to further clarify the antipneumococcal activity of clarithromycin, agar dilution MIC methodology was used to test the activities of clarithromycin, erythromycin, and azithromycin against 120 randomly chosen recent isolates of penicillin-susceptible, -intermediate, and -resistant pneumococci from the United States. In the second part of the study, six penicillin-susceptible and -resistant but macrolide-susceptible pneumococci were tested against the three compounds by broth microdilution and time-kill methodology.

A total of 120 isolates of Streptococcus pneumoniae isolated from blood, ear, nasopharynx, or sputum were examined. All strains were isolated from 10 centers throughout the United States and comprised 39 penicillin-susceptible (MICs of ≤0.06 µg/ml), 38 penicillin-intermediately resistant (MICs of 0.1 to 1.0 µg/ml) and 43 penicillin-resistant (MICs of ≥2.0 µg/ml) isolates. Strains were diverse with regard to serotype, but penicillin-intermediate and -resistant strains comprised the five most commonly encountered resistant serotypes (8). Penicillin-binding protein patterns of these strains are not all available. Antimicrobial powders were obtained from their respective manufacturers; in all cases, potencies were used to adjust for differences in antibiotic concentrations. Agar dilution MICs were determined by National Committee for Clinical Laboratory Standards methodology (12). Plates were incubated overnight in ambient air before interpretation. Quality control strains, including S. pneumoniae ATCC 49619, were included in each run.

For six macrolide-susceptible strains (two penicillin-susceptible, two penicillin-intermediate, and two penicillin-resistant strains), broth microdilution MICs were determined according to standard methodology (12). Time-kills were as described previously (14), with inocula of 5 × 10^5 to 5 × 10^6 CFU/ml and detection thresholds of 250 to 300 CFU/ml. Bacterial carry-over was dealt with as described previously (14). Viability
counts were performed at 0, 2, 4, 6, 12, and 24 h. Bactericidal activity was defined as a $3 \Delta \log_{10} \text{CFU/ml}$ drop in colony count compared with time zero, and bacteriostatic activity was defined as a 0 to 3 $\Delta \log_{10} \text{CFU/ml}$ drop in colony count compared with time zero.

Results of agar dilution MICs are presented as MICs at which 50% of the isolates were inhibited (MIC$_{50}$) and MIC$_{90}$ in Table 1. As can be seen, clarithromycin yielded MIC$_{50}$ and MIC$_{90}$ which were usually one or two dilutions lower than those of erythromycin and azithromycin.

Results of time-kill studies are presented in Table 2. It should be noted that all strains were chosen to be macrolide susceptible. Broth microdilution MICs of the six strains were 0.032 to 0.064, 0.032 to 0.125, and 0.016 to 0.064 $\mu$g/ml for erythromycin, azithromycin, and clarithromycin, respectively, with no significant differences among the S, I, and R strains. The respective bacteriostatic and bactericidal values (micrograms per milliliter) for the six strains were 0.004 to 0.03 and 0.016 to 0.03 (0.004 to 0.03/0.016 to 0.03) (clarithromycin), 0.008 to 0.06/0.016 to 0.125 (erythromycin), and 0.016 to 0.06/0.03 to 0.125 (azithromycin). All compounds were uniformly bactericidal after 24 h but were not bactericidal at 2, 4, 6, or 12 h. However, erythromycin was bactericidal at 8 times the MIC, while azithromycin and clarithromycin showed comparable activity at 2 times the MIC. All three compounds showed 90% killing of all strains after 12 h at 2 times the MIC (Table 2).

Results of the present study confirm previous findings that clarithromycin MICs for pneumococci are one or two dilutions lower than those of erythromycin and azithromycin (2, 10, 13). Additionally, results of time-kill studies show superior bactericidal activity of clarithromycin and azithromycin compared with erythromycin. The relevance of slow bactericidal activity of macrolides against pneumococci in nonmeningitic infections is unknown and requires clarification by therapeutic trials.

Recent studies have also shown that, while azithromycin has higher tissue/plasma ratios than other macrolides, the absolute concentrations of clarithromycin in alveolar macrophage cells and epithelial lining fluid is higher for up to 8 and 12 h, respectively, after the last dose (7, 15). Broad-spectrum macrolides such as azithromycin and clarithromycin are approved for use in conditions such as community-acquired lower respiratory tract infections caused by organisms such as pneumococcus, *Hemophilus influenzae*, *Brachyella catarrhalis*, *Staphylococcus aureus*, chlamydiae, *Mycoplasma pneumoniae*, and legionellas. The role of slow bactericidal activity of all macrolides against pneumococci, as well as the improved in vitro activity of clarithromycin against penicillin-susceptible and -intermediate strains, needs to be clarified by clinical studies.

The incidence of macrolide-resistant, penicillin-susceptible and -intermediate pneumococci in the U.S. isolates studied was very low in the present study. Additionally, many penicil-
lin-resistant strains were still macrolide susceptible. The present study needs to be supplemented by a larger study delineating the relationship between penicillin and macrolide susceptibility in U.S. pneumococcal strains. The results of this study indicate that clarithromycin can probably be used in the United States for therapy of nonmeningeal pneumococcal infection caused by strains which are susceptible or intermediate resistant to penicillin. In areas where penicillin-resistant strains are common, however, other therapeutic modalities must be chosen. Clinical studies will be necessary to test these hypotheses.

This study was supported by a grant from Abbott Laboratories, Chicago, Ill.

REFERENCES