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Microbiologic Efficacy of Azithromycin and Susceptibilities to Azithromycin of Isolates of *Chlamydia pneumoniae* from Adults and Children with Community-Acquired Pneumonia

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***Chlamydia pneumoniae* was eradicated from the nasopharynges of 26 of 33 (78.8%) evaluable children and adults with community-acquired pneumonia who were treated with azithromycin. We tested 55 isolates of *C. pneumoniae* obtained from 46 of these patients against azithromycin. The MIC at which 90% of the isolates were inhibited and the minimal chlamydiacidal concentration at which 90% of strains tested were killed of azithromycin for these isolates were both 0.5 μg/ml. Seven patients remained culture positive after treatment. The MICs of azithromycin for isolates from two patients increased fourfold after therapy. However, all the patients with persistent infection improved clinically. Further studies of treatment of *C. pneumoniae* infection, utilizing culture, are needed both to assess efficacy and to monitor for the possible development of antibiotic resistance.**

Chlamydia pneumoniae is a frequent cause of community-acquired respiratory tract infections, including pneumonia and bronchitis (2, 4). Although macrolides are frequently recommended as first-line drugs for treatment of *C. pneumoniae* infection, there are limited data on the use of these antibiotics. Practically all previously published treatment studies have used serology only; thus, microbiologic efficacy could not be assessed (1, 3, 11–13). One multicenter treatment study of community-acquired pneumonia in children found the efficacies of clarithromycin and erythromycin for eradicating *C. pneumoniae* from the nasopharynx to be 79 and 86%, respectively (2). Persistence of the organism was not associated with the development of antibiotic resistance in vitro (10). Azithromycin is also active against a wide range of organisms responsible for community-acquired pneumonia and has pharmacokinetics and tolerance superior to those of erythromycin. Preliminary studies from our laboratory have demonstrated that azithromycin has in vitro activity against *C. pneumoniae* similar to that of erythromycin (6). As part of two nationwide, multicenter studies, which evaluated a 5-day course of oral azithromycin for the treatment of community-acquired pneumonia in adults and children, we performed in vitro susceptibility testing of azithromycin against isolates of *C. pneumoniae* from these patients.

Adult pneumonia treatment study. Patients 12 years of age or older presenting with community-acquired pneumonia were enrolled in the study. Inclusion criteria included radiographic evidence of pneumonia, no history of allergy to macrolide antibiotics, and no serious underlying disease. This was an open, noncomparative, multicenter study evaluating 1.5 g of azithromycin given orally over 5 days. Samples from patients were cultured at baseline and at 10 to 14 days and 6 weeks after the initiation of treatment.

Pediatric community-acquired pneumonia treatment study. Children 6 months through 16 years of age presenting with community-acquire pneumonia were enrolled in the study. Inclusion criteria included radiographic evidence of pneumonia, no history of allergy to macrolide antibiotics, and no serious underlying disease. The children were randomized (2:1) to receive pediatric suspensions of either azithromycin or the comparative agent (amoxicillin-clavulanate if <5 years of age or erythromycin if ≥5 years of age). The dosage of the azithromycin suspension was 10 mg/kg of body weight once on day 1 (maximum, 500 mg) followed by 5 mg/kg once daily (maximum, 250 mg/day) on days 2 to 5. The amoxicillin-clavulanate suspension was given at a dosage of 40 mg/kg per day, in three divided doses for 10 days, and the erythromycin estolate suspension was given at a dosage of 40 mg/kg per day, in three divided doses for 10 days. Samples from patients taken at baseline and at 15 to 19 days after the initiation of treatment were cultured.

Nasopharyngeal swab specimens were obtained for *C. pneumoniae* culture. Azithromycin (Pfizer) and erythromycin were supplied as powders and were solubilized according to the instructions of the manufacturer. Culture of *C. pneumoniae* was performed at SUNY Health Science Center at Brooklyn by utilizing cycloheximide-treated HEp-2 cells grown in 96-well microtiter plates (9). After 72 h of incubation all specimens were passaged once. Cultures were confirmed by fluorescent-antibody staining with a *C. pneumoniae*-specific monoclonal antibody (Washington Research Foundation). Patient isolates were passaged five to six time in cell culture in antibiotic-free medium.

Susceptibility testing of *C. pneumoniae* was performed in cell culture by using HEp-2 cells grown in 96-well microtiter plates (6). Each well was inoculated with 0.2 ml of the organism diluted to yield 10^3 inclusion-forming units per ml, and the plates were centrifuged at 2,000 × *g* for 1 h. The wells were then aspirated and overlaid with 0.2 ml of medium containing 1 μg of cycloheximide per ml and serial twofold dilutions of the test drug. After incubation at 35°C for 72 h, cultures were fixed and stained for inclusions with fluorescein-conjugated antibody to the lipopolysaccharide antigen common to *Chlamydia* (Pathfinder Chlamydia Culture Confirmation System; Kallestad Diagnostics). The MIC was the lowest antibiotic concen-

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TABLE 1. In vitro susceptibilities to azithromycin of isolates of *C. pneumoniae* from three persistently positive adults with pneumonia

Patient no.	Date (mo/day/yr)	MIC (μg/ml)	MCC (μg/ml)
072	4/26/93	0.062	0.062
	5/5/93	0.250	0.250
	6/2/93	0.250	0.250
226	4/26/94	0.015	0.062
	5/9/94	0.015	0.015
043	3/18/94	0.250	0.015
	3/30/94	0.250	0.250
	4/28/94	0.250	0.250

tration at which no inclusions were seen. The minimal chlamydiacidal concentration (MCC) was determined by freezing the cultures at −70°C, thawing the cultures, passaging the disrupted cell monolayers onto new cells, incubating the cells for 72 h, and then fixing and staining the cells as described above. The MCC was the lowest antibiotic concentration which resulted in no inclusions after passage. All tests were run in triplicate.

C. pneumoniae was isolated from 10 of 48 (20.8%) patients with pneumonia who were enrolled in the adult study. Patients were from six sites in five states (Georgia, New York, Wisconsin, Texas, and Massachusetts) and the District of Columbia. *C. pneumoniae* was eradicated from the nasopharynges of 7 of the 10 (70%) culture-positive pneumonia patients after treatment. We were able to retrieve five additional isolates from the three persistently infected patients. The MIC at which 50% of the isolates were inhibited (MIC_{50}) and MIC_{90} for these 15 isolates were both 0.25 μg/ml (range, 0.015 to 0.25 μg/ml). The MCC_{90}s were 0.25 μg/ml (range, 0.015 to 0.25 μg/ml). The in vitro susceptibilities of the isolates from the three persistently infected patients are shown in Table 1. The MICs did not change during or after therapy except for patient 072. Both the MICs and MCCs for two isolates obtained from this patient after treatment increased fourfold, from 0.062 to 0.25 μg/ml. Despite persistence of *C. pneumoniae*, all patients improved clinically.

A total of 456 children with pneumonia from 27 sites in 20 states were enrolled in the pediatric treatment study. *C. pneumoniae* was isolated from 36 (7.9%) of the children. Two culture-positive patients did not return for any follow-up visits, leaving 34 patients for whom we could evaluate microbiologic efficacy. *C. pneumoniae* was eradicated after treatment from the nasopharynges of 19 of 23 (83%) evaluable patients who received azithromycin and 4 of 4 and 7 of 7 of those patients who received amoxicillin-clavulanate and erythromycin, respectively ($P = 0.9$, chi-square test).

MICs and MCCs of azithromycin and erythromycin for 40 isolates of *C. pneumoniae* from 36 pediatric patients were determined. The MIC_{50} and MIC_{90} of azithromycin were 0.125 and 0.5 μg/ml (range, 0.015 to 0.5 μg/ml), respectively. The MIC_{50} and MIC_{90} of erythromycin were 0.062 and 0.25 μg/ml (range, 0.015 to 0.5 μg/ml), respectively. As shown in Table 2, the MICs and MCCs for the isolates from one of four persistently infected children (patient 015) increased fourfold, from 0.031 to 0.125 μg/ml for both azithromycin and erythromycin.

The efficacies of azithromycin for eradication of *C. pneumoniae* from the nasopharynges of adults and children with pneumonia were 70% (7 of 10) and 83% (19 of 23), respectively. Overall, *C. pneumoniae* was eradicated from the nasopharynges of 26 of the 33 (78.8%) patients who were treated with azithromycin. The numbers of children treated with the drugs used for comparison were too small to make any meaningful comparisons. Persistence did not appear to be related to poor compliance by the adults or the children. The parents brought back the bottles of suspension, which were weighed, and the amount of drug actually given was calculated. These results are comparable to those of our previous study of the treatment of *C. pneumoniae* pneumonia in children, in which we found a microbiologic efficacy of 79% (15 of 19) for clarithromycin and 86% (12 of 14) for erythromycin (2). There are no published data on the microbiological efficacy of azithromycin for treatment of *C. pneumoniae* infections in adults.

Few published data on the efficacy of any treatment regimen for eliminating *C. pneumoniae* from the respiratory tract exist. We have observed several patients who have remained persistently culture positive and clinically symptomatic despite 7- to 30-day courses of doxycycline, tetracycline, and erythromycin (5). There are no published pneumonia treatment studies that have assessed the efficacy of azithromycin for the treatment of *C. pneumoniae* infection by utilizing culture (11–13). All have relied on serology for diagnosis, which has a very poor correlation with culture positivity, especially in children (2). A recent study of children compared the efficacy of a 3-day course of azithromycin to 10 days of erythromycin for the treatment of community-acquired pneumonia (11). Although it was stated in that study that serology for *C. pneumoniae* was determined by the microimmunofluorescence method, no data on the number of children who had serologic evidence of infection were presented, which suggests that perhaps none were positive. Block et al. found that 77% of the children in their study with culture-positive *C. pneumoniae* pneumonia were antibody negative by microimmunofluorescence (2).

The MICs and MCCs of azithromycin for the strains isolated from the patients in these two studies were similar to what we have previously reported (6). There was no difference in susceptibility between the strains isolated from adults and children. We previously reported the MIC_{90} and MCC_{90} of azithromycin to be 0.125 and 0.25 μg/ml, respectively (6). Only 11 strains, primarily from adults from Brooklyn, N.Y., were tested in that study. None of those patients were treated with azithromycin. The data presented here are the result of the first attempt to correlate the results of treatment with azithromycin and in vitro susceptibility with a large number of strains from diverse geographical areas. The MICs and MCCs for three of nine isolates obtained after treatment from two of

TABLE 2. MICs and MCCs for isolates of *C. pneumoniae* from four persistently positive children with pneumonia treated with azithromycin[a]

Patient no.	Date (mo/day/yr)	Azithromycin		Erythromycin	
		MIC	MCC	MIC	MCC
015	7/8/94	0.031	0.031	0.031	0.062
	7/26/94	0.125	0.125	0.125	0.25
1321	12/7/94	0.5	0.5	0.25	0.25
	12/21/94	0.5	0.5	0.25	0.25
356	2/8/94	0.25	0.5	0.125	0.125
	3/22/94	0.25	0.25	0.125	0.125
032	2/1/94	0.25	0.25	0.125	0.125
	2/16/94	0.125	0.25	0.062	0.062

[a] All concentrations are in micrograms/milliliter.

seven persistently infected patients who were treated with azithromycin increased fourfold after treatment, although they were still within the range considered to indicate susceptibility to the antibiotic. It is not clear if this is an isolated event or suggestive of possible development of resistance. All patients improved clinically despite persistence of the organism. In our previous experience with the use of clarithromycin and erythromycin we did not find any change in MIC or MCC despite persistence of the organism in eight children with pneumonia, two of whom were treated with erythromycin and six of whom were treated with clarithromycin (10). The results of in vitro susceptibility testing may not always predict in vivo efficacy. Although relative resistance of *Chlamydia trachomatis* to erythromycin and doxycycline has been reported, the relationship to treatment failure is unclear (7, 8). Further studies of treatment of *C. pneumoniae* infection, utilizing culture, are needed both to assess efficacy and to monitor for the possible development of antibiotic resistance.

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