ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1994, p. 1588–1589
0066-4804/94/$04.00+0
Copyright © 1994, American Society for Microbiology
Vol. 38, No. 7

Susceptibilities to Clarithromycin and Erythromycin of Isolates of *Chlamydia pneumoniae* from Children with Pneumonia

PATRICIA M. ROBLIN, GLORIA MONTALBAN, AND MARGARET R. HAMMERSCHLAG*

Department of Pediatrics, Division of Infectious Diseases, SUNY Health Science Center at Brooklyn, Brooklyn, New York 11203-2098

Received 9 March 1994/Returned for modification 20 April 1994/Accepted 13 May 1994

We tested in vitro 49 isolates of *Chlamydia pneumoniae* obtained from 35 children with community-acquired pneumonia against clarithromycin and erythromycin. The children were part of a treatment study comparing the two drugs. Clarithromycin was 2- to 10-fold more active than erythromycin, with a MIC for 90% of strains tested and minimal chlamydiacidal concentration for 90% of strains tested of 0.031 μg/ml compared with 0.125 μg/ml for erythromycin. Eight of these children, two of whom were treated with erythromycin and six of whom received clarithromycin, remained culture positive after treatment. We were able to test 21 isolates from these children. All were susceptible to both drugs, and the MICs did not change after therapy.

Chlamydia pneumoniae is emerging as a frequent cause of community-acquired respiratory tract infection in adults and children, including pneumonia and bronchitis (3, 4, 7). There are limited data on the treatment of these infections, because most studies have used serology only, and thus microbiologic efficacy could not be assessed. As part of a nationwide, multicenter study comparing clarithromycin and erythromycin suspensions for the treatment of community-acquired pneumonia in children 3 through 12 years of age, we isolated *C. pneumoniae* from 42 (16%) of the 260 children enrolled (7). Although all of the children with *C. pneumoniae* infection improved clinically and radiographically, 9 (21%) of these children remained culture positive after treatment. *C. pneumoniae* was eradicated from 15 of 19 (79%) evaluable patients who were treated with clarithromycin and 12 of 14 (86%) of those treated with erythromycin. The dosages of clarithromycin and erythromycin suspensions were 15 and 40 mg/kg of body weight per day, respectively, both given in two divided doses for 10 days. We performed in vitro susceptibility testing against clarithromycin and erythromycin with isolates of *C. pneumoniae* from these children.

MATERIALS AND METHODS

Clarithromycin (Abbott Laboratories, North Chicago, Ill.) and erythromycin (Eli Lilly and Co., Indianapolis, Ind.) were supplied as powders and solubilized according to the instructions of the manufacturers.

The *C. pneumoniae* isolates were originally isolated from nasopharyngeal swab specimens cultured in cycloheximide-treated HEp-2 cells (1). Patient isolates were passed three to four times in tissue culture in antibiotic-free medium.

Susceptibility testing of *C. pneumoniae* was performed with cell culture of 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/ml and centrifuged at 2,000 × *g* for 1 h. The wells were then aspirated and overlayed with 0.2 ml of medium (Iscove's modified Dulbecco's medium [GIBCO]) 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 genus antigen (Pathfinder Chlamydia Culture Confirmation System; Kallestad Diagnostics, Chaska, Minn.).

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

RESULTS

We were able to retrieve 49 isolates from 35 of the 42 children with pneumonia who had positive *C. pneumoniae* cultures; the MICs and MCCs are shown in Table 1. Overall, clarithromycin was 2- to 10-fold more active than erythromycin, with a MIC for 90% of the strains tested (MIC_{90}) and MCC for 90% of the strains tested (MCC_{90}) of 0.031 μg/ml compared with 0.125 μg/ml for erythromycin. Twenty-four of the *C. pneumoniae*-positive children were treated with clarithromycin, and 18 were treated with erythromycin. Nine of the children with *C. pneumoniae* infection remained culture positive after treatment; seven of these children were treated with clarithromycin and two received erythromycin. We were able to retrieve 21 isolates from eight of these patients, including two children who were treated with erythromycin and six who received clarithromycin. Both antibiotics were given for 10 days. The isolates were obtained at the baseline visit before treatment, within 48 h after the end of treatment, and 4 to 6 weeks after treatment. The MICs for these isolates are shown in Table 2. All of these isolates were susceptible to both antibiotics, and the MICs did not change during or after therapy. Despite the persistence of *C. pneumoniae*, all of these patients improved clinically with complete resolution of the infiltrates shown on their chest radiographs.

DISCUSSION

Doxycycline and erythromycin are considered the treatment of choice for infections due to *C. trachomatis* and by extrapolation have been recommended for the treatment of infections

* Corresponding author. Mailing address: Department of Pediatrics, Box 49, SUNY Health Science Center at Brooklyn, 450 Clarkson Ave., Brooklyn, NY 11203-2098. Phone: (718) 245-4075. Fax: (718) 245-2118.

TABLE 1. In vitro susceptibilities of 49 isolates of *C. pneumoniae* from 35 children with community-acquired pneumonia

Drug	MIC (μg/ml)[a]			MCC (μg/ml)[b]	
	Range	50%	90%	Range	90%
Clarithromycin	0.004–0.25	0.016	0.031	0.004–0.25	0.031
Erythromycin	0.016–0.125	0.065	0.125	0.016–0.25	0.125

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.
[b] 90%, MCC_{90}.

due to *C. pneumoniae*. Several of the original patients with *C. pneumoniae* infection reported by Grayston et al. (4) were thought to have infection with *Mycoplasma pneumoniae* and were treated with erythromycin (1 g/day for 5 to 10 days). Many of these patients did not appear to respond because they had continuing or recurring symptoms; however, follow-up cultures were not done. We have observed several patients with *C. pneumoniae* infection who have been persistently culture positive and symptomatic despite 7- to 30-day courses of doxycycline and tetracycline treatment (5). Although resistance of *C. trachomatis* to tetracycline and erythromycin has been reported in vitro, the relationship to treatment failure is unclear (8, 9). Resistance of *C. pneumoniae* to tetracyclines or macrolides has not yet been described.

TABLE 2. MICs for isolates of *C. pneumoniae* from 8 persistently positive patients

Patient	Treatment	Date (mo/day/yr)[a]	MIC of:	
			Clarithromycin	Erythromycin
264	Erythromycin	2/2/93	0.008	0.031
		2/16/93	0.008	0.031
543	Erythromycin	3/24/93	0.031	0.065
		4/5/93	0.031	0.065
		5/4/93	0.031	0.065
458	Clarithromycin	3/24/93	0.016	0.031
		4/5/93	0.016	0.031
		5/10/93	0.016	0.031
237	Clarithromycin	11/5/92	0.016	0.065
		12/17/92	0.016	0.125
473	Clarithromycin	9/11/92	0.25	0.125
		10/19/92	0.25	0.125
519	Clarithromycin	2/3/93	0.016	0.125
		2/19/93	0.031	0.125
		3/22/93	0.031	0.125
166	Clarithromycin	3/4/93	0.016	0.125
		3/15/93	0.016	0.125
		4/19/93	0.016	0.125
364	Clarithromycin	5/30/92	0.016	0.031
		6/11/92	0.031	0.031
		7/16/92	0.031	0.065

[a] Date on which the strain was isolated from the patient.

The MICs and MCCs obtained in this study were similar to those in our earlier study of 11 strains which found a MIC_{90} and MCC_{90} of 0.031 μg/ml (6). The range was 0.004 to 0.031 μg/ml, compared with 0.004 to 0.25 μg/ml in the present study. As we examined a substantially larger number of strains, we began to see more interstrain variation. Only one of the isolates from the 35 patients required a MIC of 0.25 μg/ml. Clarithromycin is one of the most active antibiotics against *C. pneumoniae* in vitro (6).

Clarithromycin has excellent tissue and intracellular penetration, especially into bronchial epithelium and alveolar macrophages (2). These observations suggest that clarithromycin should be superior to erythromycin for the treatment of respiratory infections due to *C. pneumoniae*; however, the results of the pediatric pneumonia treatment study suggest that the two drugs are equivalent in terms of eradicating *C. pneumoniae* from the respiratory tract (1). These data also infer that performance in vitro may not predict microbiologic efficacy in vivo. Even though the organism persisted after treatment in nine patients, they all improved clinically. The results of this study suggest that persistence of *C. pneumoniae* in these patients was not secondary to the development of antibiotic resistance.

REFERENCES

1. **Bloch, S., J. Hedrick, M. R. Hammerschlag, G. H. Cassell, and J. C. Craft.** Comparative safety and efficacy of clarithromycin and erythromycin ethyl succinate suspensions in the treatment of children with community-acquired pneumonia. Pediatr. Infect. Dis. J., in press.
2. **Fraschini, F., F. Scaglione, G. Pintucci, G. Maccarinelli, S. Dugnani, and G. Demartini.** 1991. The diffusion of clarithromycin and roxithromycin into nasal mucosa, tonsil and lung in humans. J. Antimicrob. Chemother. **27**(Suppl. A):61–65.
3. **Grayston, J. T., L. A. Campbell, C.-C. Kuo, C. H. Mordhorst, P. Saikku, D. H. Thom, and S. P. Wang.** 1990. A new respiratory tract pathogen: *Chlamydia pneumoniae* strain TWAR. J. Infect. Dis. **161**:618–625.
4. **Grayston, J. T., C.-C. Kuo, S.-P. Wang, and J. Altman.** 1986. A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infections. N. Engl. J. Med. **315**:161–168.
5. **Hammerschlag, M. R., K. Chirgwin, P. M. Roblin, M. Gelling, W. Dumornay, L. Mandel, P. Smith, and J. Schachter.** 1992. Persistent infection with *Chlamydia pneumoniae* following acute respiratory illness. Clin. Infect. Dis. **14**:178–182.
6. **Hammerschlag, M. R., K. K. Qumei, and P. M. Roblin.** 1992. In vitro activities of azithromycin, clarithromycin, L-ofloxacin, and other antibiotics against *Chlamydia pneumoniae*. Antimicrob. Agents Chemother. **36**:1573–1574.
7. **Hammerschlag, M. R., P. M. Roblin, G. Cassell, L. Duffy, R. G. Rank, S. Cox, and R. Palmer.** 1993. Role of infection with *Chlamydia pneumoniae* (Cp) in children with community acquired pneumonia (CAP) in the USA, abstr. 1377, p. 371. Program Abstr. 33rd. Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, D.C.
8. **Jones, R. B., B. Van Der Pol, D. H. Martin, and M. K. Shepard.** 1990. Partial characterization of *Chlamydia trachomatis* isolates resistant to multiple antibiotics. J. Infect. Dis. **162**:1309–1315.
9. **Mourad, A., R. L. Sweet, N. Sugg, and J. Schachter.** 1980. Relative resistance to erythromycin in *Chlamydia trachomatis*. Antimicrob. Agents Chemother. **18**:696–698.