

In Vitro and In Vivo Activities of Azithromycin, a New Azalide Antibiotic, against Chlamydia

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The in vitro and in vivo activities of azithromycin against chlamydia were investigated. The MIC of azithromycin for five standard strains of different species of chlamydia and six wild-type strains of *Chlamydia pneumoniae* was 0.125 µg/ml, which was superior to that of erythromycin but inferior to those of clarithromycin and minocycline. However, the therapeutic effect of a 7-day course of azithromycin at a dose of 10 mg/kg of body weight administered orally once daily to mice with experimental *Chlamydia psittaci* pneumonia was excellent, with a 100% survival rate at 14 days after infection, which was the same as that for treatment with minocycline administered at 10 mg/kg twice daily; all erythromycin treated animals died within 10 days. When treatment was discontinued 3 days after the infection, the survival rate for mice treated with azithromycin was 90% and that for mice administered minocycline was 30%. These results suggest that azithromycin may be useful in the treatment of respiratory infections caused by intracellular pathogens, including chlamydia because of its excellent accumulation within host cells.

Azithromycin is the prototype of a new class of antibiotics known as azalides and differs structurally from erythromycin because of the presence of a methyl-substituted nitrogen at the 9a position in its lactone ring. It is characterized by high and sustained concentrations in tissue and a long half-life in tissue, in addition to potent and broad antimicrobial activity (5, 10, 13, 15). Azithromycin penetrates cells and accumulates intracellularly, which suggest that it would be useful in the treatment of infections caused by intracellular pathogens such as mycobacteria, chlamydia, and *Legionella pneumophila* (6, 7, 14, 20). In the study described here the in vitro and in vivo activities of azithromycin against chlamydia were investigated and compared with those of conventional antibiotics.

MATERIALS AND METHODS

Measurement of MICs. MICs were measured by the standard methods of the Japan Society of Chemotherapy (11). Briefly, HeLa 229 cells were seeded into 24-well plates 24 h prior to chlamydial inoculation. Cell monolayers were examined for confluency and were inoculated with 10⁴ inclusion-forming units (IFU) of various chlamydia strains per well.

The IFU were determined by the following method. A serially diluted suspension containing chlamydia was inoculated onto confluent monolayers of HeLa 229 cells.

After incubation, the number of inclusion bodies was counted. The number of IFU per milliliter was calculated on the basis of the number of inclusion bodies, inoculum size, and fold dilution of the suspension.

The standard strains of chlamydia tested were *C. psittaci* Budgerigar (obtained from the Japan National Institute of Health, Tokyo) and California 10 (obtained from A. Matsumoto, Department of Microbiology, Kawasaki Medical School), *C. trachomatis* D/UW-3/Cx strain (obtained from the Washington Research Foundation, Seattle), and *C. pneu-*

moniae TW-183 and AR-39 (obtained from the Washington Research Foundation). The MICs for six strains of *C. pneumoniae* isolated from patients with acute respiratory infections and high *C. pneumoniae* antibody titers were also examined.

After inoculation, culture medium (Eagle minimal essential medium supplemented with 10% fetal bovine serum, 1 µg of cycloheximide per ml, and antibiotics) diluted with the test compound was applied, and the plates were incubated at 37°C in 5% CO₂ for 48 h (*C. psittaci*), 37°C in 5% CO₂ for 72 h (*C. trachomatis*), and 35°C in 5% CO₂ for 72 h (*C. pneumoniae*) prior to staining of the inclusions.

Cultureset (Ortho Diagnostic Systems Co. Ltd., Tokyo, Japan), a genus-specific fluorescein isothiocyanate-conjugated monoclonal antibody to chlamydial lipopolysaccharide, was used to stain the inclusions.

The antibiotics tested were azithromycin (a gift from Pfizer Pharmaceutical Co. Ltd.), clarithromycin, roxithromycin, erythromycin, minocycline, ofloxacin, tosufloxacin, and ciprofloxacin.

Therapeutic effect in mice with *C. psittaci* pneumonia. Cells infected with *C. psittaci* California 10 were micronized by ultrasonic treatment and were diluted with sucrose-phosphate-glutamic medium to an appropriate titer. Then, 5-week-old male MCH mice were infected with the cell solution (10⁵ IFU per animal) by nasal instillation.

The animals were divided into four groups (10 animals in each group), and at 24 h after the infection, oral administration of antibiotics was started. These antibiotics were azithromycin, minocycline, and erythromycin. They were suspended in 5% gum arabic and were administered at a dose of 10 mg/kg of body weight once daily for azithromycin and at 10 mg/kg twice daily for minocycline and erythromycin. Control mice received only 5% gum arabic.

Animals were treated for 3 or 7 days. They were monitored every day for 14 days to determine the survival rates and to compare the therapeutic effects of each drug.

Statistical analyses were done by the log-rank test for comparison of the survival times and Fisher's exact test for the survival rates at 14 days.

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TABLE 1. MICs of azithromycin in comparison with those of other antibiotics for standard strains of *Chlamydia* spp.

Antibiotics	MIC ($\mu\text{g/ml}$)				
	<i>C. psittaci</i>		<i>C. trachomatis</i> D/UW-3/Cx	<i>C. pneumoniae</i>	
	Budgerigar	California 10		TW-183	AR-39
Azithromycin	0.125	0.125	0.125	0.125	0.125
Clarithromycin	0.015	0.015	0.015	0.015	0.015
Roxithromycin	0.125	0.125	0.25	0.125	0.063
Erythromycin	0.25	0.25	0.5	0.25	0.125
Minocycline	0.015	0.015	0.015	0.015	0.015
Ciprofloxacin	1		0.5	1	
Ofloxacin	0.5	0.5	0.5	0.5	1
Tosufloxacin	0.063	0.063	0.125	0.125	

RESULTS

MICs. The MIC of azithromycin for standard strains of three different species of chlamydia and six wild-type strains of *C. pneumoniae* was 0.125 $\mu\text{g/ml}$ (Tables 1 and 2).

Clarithromycin showed excellent activity at a MIC of 0.015 $\mu\text{g/ml}$, which was equal to that of minocycline. There was no significant difference in the MICs of each drug for the different chlamydia species.

Therapeutic Effects in Mice with *C. psittaci* Pneumonia. As shown in Fig. 1, the survival rates for mice treated with azithromycin or minocycline for 7 days was 100%, while all control animals and erythromycin-treated animals died within 7 and 10 days of the initiation of treatment, respectively.

In the group receiving a 3-day course of therapy, 90% of azithromycin-treated mice had survived by day 14. These survival rates or times were significantly longer than those obtained with minocycline (30%, $P = 0.02$ and $P = 0.0042$ for survival time and survival rate, respectively) or erythromycin

TABLE 2. MICs of azithromycin for six wild strains of *C. pneumoniae*

Antibiotic	MIC ($\mu\text{g/ml}$) for <i>C. pneumoniae</i> strain:					
	KKpn-2	KKpn-3	KKpn-4	KKpn-5	KKpn-6	KKpn-15
Azithromycin	0.125	0.125	0.125	0.125	0.125	0.125
Erythromycin	0.125	0.125	0.125	0.125	0.125	0.125
Minocycline	0.015	0.015	0.015	0.015	0.015	0.015

(10%; $P < 0.001$ for both tests) and the control group (0%; $P < 0.001$ for both tests) (Fig. 2).

DISCUSSION

Azithromycin is the first azalide antibiotic; its unusual pharmacokinetic characteristics and potent antimicrobial activities have been reported previously (4-6, 10, 14, 17). The properties of azithromycin promised that it would have a high degree of efficacy in the treatment of respiratory infections, especially those caused by intracellular pathogens (3, 6). The results of many preclinical and clinical trials have suggested the usefulness of a short course of administration of this drug to patients with bacterial and nonbacterial respiratory infections, including chlamydial infections (2, 3, 13, 19).

Chlamydia species are well-known respiratory pathogens that cause upper and lower respiratory tract infections and pneumonia. *C. trachomatis* causes pneumonia in newborns, *C. psittaci* is known as a causative organism of psittacosis, and *C. pneumoniae* is a newly recognized chlamydia species which has been shown to be an important pathogen of acute respiratory infections worldwide (8).

Some of the recently developed antibiotics, such as new quinolones and new macrolides, show excellent antichlamydial activities in vitro and in vivo (12, 18). This is one of the

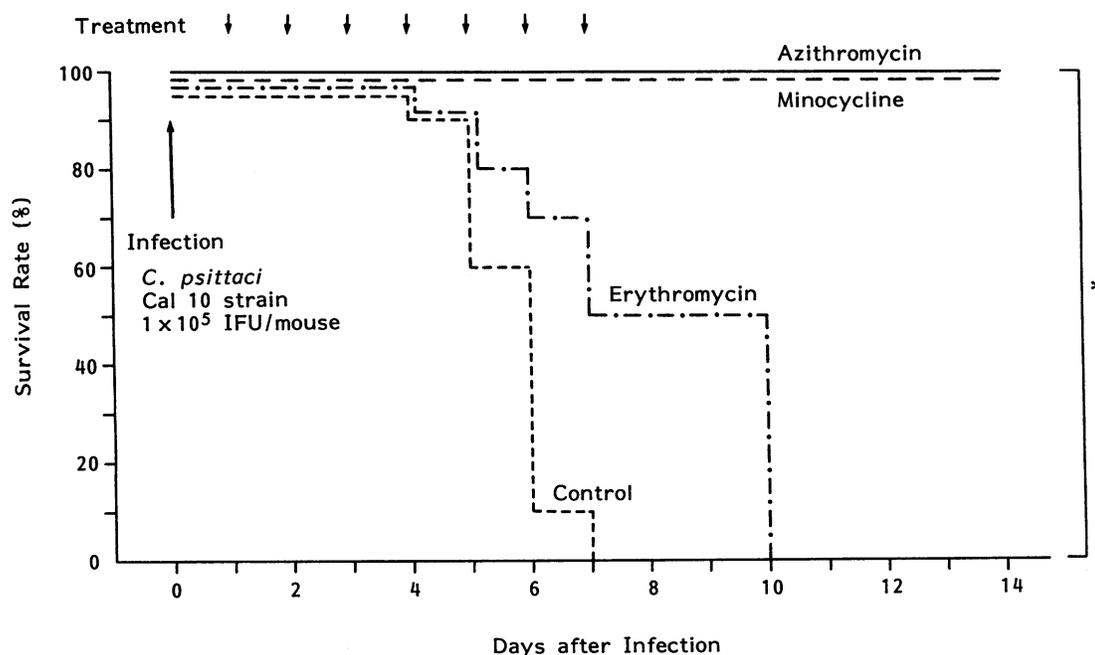


FIG. 1. Therapeutic effects of azithromycin against mouse *C. psittaci* pneumonia. Treatment was started 24 h after the infection with 10 mg of azithromycin per kg once daily or 10 mg of minocycline or erythromycin per kg twice daily for 7 days. ($n = 10$ mice). *, $P < 0.001$ (both tests).

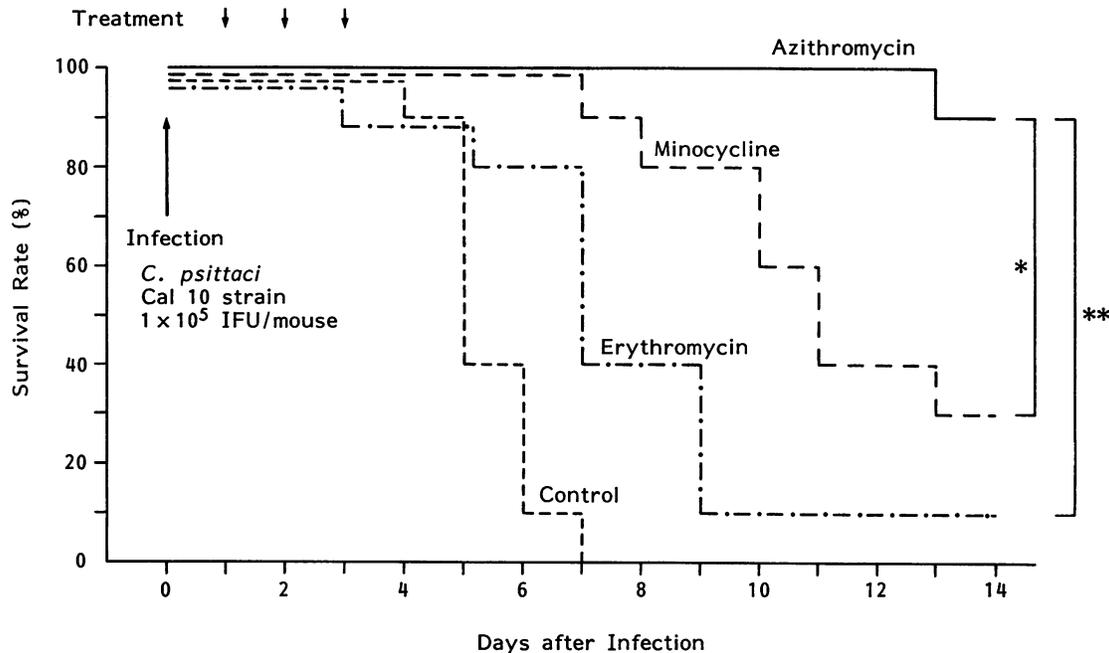


FIG. 2. Therapeutic effects of azithromycin in mouse *C. psittaci* pneumonia. Treatment was started 24 h after the infection with 10 mg of azithromycin per kg once daily or with 10 mg of minocycline or erythromycin per kg twice daily for 3 days ($n = 10$ mice). *, $P < 0.042$ (survival time) and $P = 0.02$ (survival rate). **, $P < 0.001$ (both tests).

important factors of oral antibiotics as first-choice drugs for the treatment of acute respiratory tract infections.

In our study as well, these new oral agents (ofloxacin, ciprofloxacin, clarithromycin, and roxithromycin) had very low MICs; MICs were equal or much lower than that of azithromycin. These in vitro activities of azithromycin and other agents have been described in previous reports (1, 9, 21).

However, in experiments with animals, we found that the therapeutic effect of azithromycin against *C. psittaci* pneumonia was excellent and was equal to those of clarithromycin and roxithromycin (16). In the present study, only mice treated with azithromycin or minocycline survived after 7 days of treatment. Moreover, the dose of azithromycin required to attain 100% survival was half the dose of minocycline. Even in the case of animals treated for only 3 days, 90% of those treated with azithromycin were alive at day 14. However, only 30% of mice treated with minocycline for 3 days survived.

We have previously reported the therapeutic effects of new quinolones, including those of ofloxacin and tosufloxacin, in the same model of mouse *C. psittaci* pneumonia (12). While all animals treated with 20 to 40 mg of minocycline per kg/day for 7 days survived by day 7, only 53 and 60% of those treated with ofloxacin and tosufloxacin, even at a dose of 40 mg/kg twice daily for 7 days, survived by the end of treatment.

These discrepancies between the in vitro MICs and survival rates suggest the importance of the pharmacokinetic features of each agent in clinical use.

The results of the present study indicate that it is possible to achieve satisfactory clinical results in the treatment of chlamydial respiratory tract infections and other infections caused by intracellular pathogens with a relatively short course of azithromycin administered once daily because of its excellent accumulation within host cells.

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