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Prophylactic and Therapeutic Activities of Azithromycin in a Mouse Model of Pneumococcal Pneumonia

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Azithromycin is a new acid-stable 15-membered-ring macrolide that exhibits an extended half-life and excellent tissue distribution, including distribution in the lung. We compared its in vivo activity with that of erythromycin using two models of *Streptococcus pneumoniae* pneumonia, namely, a model of acute infection in Swiss mice and a model of subacute infection in C57BL/6j mice. Female mice were infected by oral delivery into the trachea of 10^5 CFU of a virulent serotype 3 strain of *S. pneumoniae* (P 4241). Prophylactic and therapeutic treatments were given orally (p.o.) or subcutaneously (s.c.) by various regimens. In the model of subacute infection, a single dose of azithromycin, 25 mg/kg, given p.o. 7 h before infection protected 92% of the mice, while erythromycin was completely ineffective. In the model of acute infection, a single dose of azithromycin, 50 mg/kg, given s.c. 24 h prior to challenge protected 80% of the mice, whereas only 35% of the mice survived with erythromycin, 50 mg/kg, 1 h before challenge. Therapy, which was studied exclusively in the model of subacute infection, was initiated 48 h postinfection. Two doses of 12.5 mg/kg given p.o. 12 h apart resulted in 80% survival of mice treated with azithromycin versus 7% survival of mice treated with erythromycin. Pulmonary clearance of bacteria was consistent with the survival rates. Two doses (25 mg/kg) of azithromycin given s.c. at 48 and 65 h after infection led to complete clearance of bacteria from the lungs and blood, whereas erythromycin-treated mice remained bacteremic. The pharmacokinetics of azithromycin account for its superior efficacy against *S. pneumoniae* pneumonia relative to the efficacy of erythromycin.

Azithromycin, the first member of a new class of antibiotics known as azalides, differs structurally from erythromycin by the presence of a methyl-substituted nitrogen at position 9a in the macrolide ring. This modification confers significant advantages to azithromycin. First, it has better stability than erythromycin in the presence of acidic pH (2), leading to good digestive absorption. Second, it has remarkable pharmacokinetic properties, including a more prolonged serum half-life, a better tissue distribution with high peak levels, and a longer mean residence time than erythromycin (4). In addition, the in vitro antimicrobial spectrum of azithromycin includes better activity than that of erythromycin against gram-negative organisms, particularly *Haemophilus influenzae* (6, 9, 10). However, the in vitro activity of azithromycin against *Streptococcus pneumoniae* is lower than that of erythromycin.

S. pneumoniae remains the leading cause of community-acquired pneumonia (8, 13). Because of the medical importance of this pathogen, we assessed the efficacy of azithromycin in vivo to determine whether the less potent in vitro activity was compensated by its excellent pharmacokinetic profile. The activity of azithromycin was compared with that of erythromycin in models of both acute and subacute infections of *S. pneumoniae* disease. Prophylactic and therapeutic regimens were studied, and antibiotic efficacies were compared in terms of survival data. Clearance of bacteria from lungs and pharmacokinetic data obtained in both noninfected and infected mice were used to evaluate the possibility of once-a-day dosing of azithromycin in a short schedule.

(Part of this work was presented at the 6th International Congress for Infectious Diseases [1a].)

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MATERIALS AND METHODS

Animals. Female C57BL/6 mice (body weight, 18 to 20 g) and Swiss mice (body weight, 20 to 22 g) were obtained from Iffa-Credo Laboratories, L'Abresle, France.

Challenge organism. Pneumococcal pneumonia was induced in mice with a serotype 3 strain (P 4241) originally isolated from blood culture and provided by the Centre de Référence du Pneumocoque (P. Geslin, Créteil, France). Virulence was maintained by monthly passage in mice, and the organism was stored in liquid nitrogen.

Experimental pneumococcal pneumonia in mice. Pneumococcal pneumonia was induced in female mice, as described in detail elsewhere (1). Briefly, animals were anesthetized by intraperitoneal injection of sodium pentobarbital, and then each mouse was infected with approximately 10^5 logarithmic-phase CFU of P 4241 by delivering 40 μl of an appropriate dilution through the mouth into the trachea. The animals were held in a vertical position for 5 min to facilitate distal alveolar migration of the bacteria by gravity. Swiss mice developed acute pneumonia and died within 3 to 4 days, with peak mortality (70 to 80%) occurring on the second day after challenge; C57BL/6 mice developed subacute pneumonia, and died within 8 to 10 days postchallenge, with peak mortality occurring on day 5 after infection. All the animals quickly became bacteremic. Since progressive pneumonia occurred and the bacterial population in the lung increased slowly in the C57BL/6 mice, this model provided an opportunity to study the action of the antibiotics at various stages of the disease. Death occurred in both models when the bacterial population exceeded 10^8 CFU per lung.

Antibiotics. The drugs studied were azithromycin (Pfizer Laboratories, Groton, Conn.) and erythromycin lactobionate (Abbott Laboratories, North Chicago, Ill.). Suspensions

of azithromycin, which was used in a free-base form, were prepared by homogenizing the powder in a standard diluent (4) containing Methocel 15 cP (0.5 g), polysorbate 80 (Tween 80) (1.0 g), carboxymethyl cellulose (low viscosity; 10.0 g), sodium chloride (9.0 g), and water (984 ml). Erythromycin lactobionate was diluted in water.

Pharmacokinetic studies in mice. The pharmacokinetic parameters of both study drugs were examined simultaneously in healthy control and infected Swiss mice. Concentrations of azithromycin and erythromycin in lungs and sera were determined after a single subcutaneous (s.c.) or oral (p.o.) dose of 50 mg/kg of body weight. In infected mice, the antibiotic was given 18 h after infection. Serum and lung samples were collected from groups of three to six mice each at 0.5, 1, 2, 4, 8, 24, 48, 72, and 96 h following drug administration. Animals were killed with CO_2 and exsanguinated by intracardiac puncture. Blood samples were centrifuged and serum was collected. Lungs were harvested from exsanguinated mice, washed in sterile water, weighed, and then homogenized in 1 ml of phosphate buffer (pH 7.8). Homogenates were centrifuged, and supernatants were used for the assay. Antibiotic concentrations were determined by the agar well diffusion method of bioassay by using *Micrococcus luteus* ATCC 9341 as the bioassay organism and antibiotic medium 11 (Difco Laboratories, Detroit, Mich.) as the growth medium. The sensitivity of the assay was about 0.1 μg/ml or μg/g, and the relative error of the assay was less than 10%. Pharmacokinetic analyses were performed by routine graphical methods, and parameters were estimated by standard methods (7). C_{max} was the maximal concentration or activity observed, T_{max} was the time to C_{max}, $t_{1/2}$ was the elimination half-life calculated by using linear least-squares regression, and AUC_{0-96} was the area under the curve from 0 to 96 h calculated by using the trapezoidal rule method.

In vivo protection studies. The prophylactic activities of single-dose treatments with azithromycin and erythromycin were compared by using a s.c. dose of 50 mg/kg in the model of acute infection and a p.o. dose of 25 mg/kg in the model of subacute infection. Azithromycin was given from 48 to 5 h preinfection, and erythromycin was given from 8 to 1 h preinfection. Therapeutic regimens were studied exclusively in the model of subacute infection. Both drugs were given at 12-h intervals, starting at 48 h postinfection (when the experimental pneumonia was well developed). Various doses (12.5 to 50 mg/kg) of azithromycin and erythromycin were given p.o. or s.c. in single or multiple (two to four) administrations. Treatment schedules are presented with the results. In all experiments, 15 animals were used per treatment group, and all animals within the same experiment were infected simultaneously. The antibiotics were given in 0.5 ml of diluent; controls received the same volume of isotonic saline. Cumulative survival rates were recorded daily and compared.

Clearance of bacteria from lung and blood. The total recoverable CFU from whole-lung homogenates and intracardiac blood samples were determined. Both azithromycin and erythromycin were given s.c. at 25 mg/kg per injection. Samples were obtained from the following three treatment groups to determine recoverable CFU: (i) untreated control mice, (ii) treated mice receiving a single injection 48 h after infection, and (iii) treated mice receiving injections at 48 and 65 h postinfection. Detailed schedules are given with the results.

Statistics. Survival rate data were analyzed by Fisher's exact test. CFU values were compared by analysis of variance; the degree of significance was determined by Snedecor's F test with the appropriate degree of freedom. When the F value was significant, each treatment group was compared with the control group and with each of the other treatment groups by using Student's t test. P values of ≤ 0.05 were considered significant.

TABLE 1. Pharmacokinetic parameters of azithromycin and erythromycin in noninfected control and infected Swiss mice following an s.c. injection of 50 mg/kg[a]

Mice and drug	Site	C_{max} (μg/ml or μg/g)[b]	T_{max} (h)	$t_{1/2}$ (h)	AUC (μg · h/ml or μg · h/g)
Noninfected control					
Azithromycin	Serum	4.7 ± 0.4	2	7.3	32
	Lung	36.3 ± 14.2	2	17.1	676
Erythromycin	Serum	5.9 ± 2.4	0.5	0.8	12
	Lung	16.1 ± 1.9	1	0.8	36
Infected					
Azithromycin	Serum	5.0 ± 1.4	1	8.7	30
	Lung	58.1 ± 6.5	2	18.6	1,041
Erythromycin	Serum	7.1 ± 0.8	0.5	0.8	12
	Lung	17.2 ± 4.2	0.5	0.9	34

[a] Values are calculated from the mean concentrations in serum and lung tissue taken at 0.5, 1, 2, 4, 8, 24, 48, 72, and 96 h postdosing.
[b] Values are means ± standard deviations for three samples.

RESULTS

Pharmacokinetics in sera and lungs. Relevant pharmacokinetic data for azithromycin and erythromycin in sera and lungs following a single 50-mg/kg s.c. or p.o. dose in noninfected and infected Swiss mice are given in Tables 1 and 2.

When the antibiotic was administered s.c. (Table 1) to healthy controls, azithromycin exhibited a prolonged $t_{1/2}$ in the sera of noninfected mice ($t_{1/2}$ = 7.3 h), and measurable levels of drug were still present in serum at 48 h postdose.

TABLE 2. Pharmacokinetic parameters of azithromycin and erythromycin in noninfected control and infected Swiss mice following an oral dose of 50 mg/kg[a]

Mice and drug	Site	C_{max} (μg/ml or μg/g)[b]	T_{max} (h)	$t_{1/2}$ (h)	AUC (μg · h/ml or μg · h/g)
Noninfected control					
Azithromycin	Serum	2.6 ± 0.2	2	4.4	20
	Lung	40.6 ± 3.7	2	17.9	697
Erythromycin	Serum	4.9 ± 0.6	1	0.7	8
	Lung	17.6 ± 9.6	1	1.1	39
Infected					
Azithromycin	Serum	3.0 ± 0.4	2	7.6	23
	Lung	34.0 ± 4.9	2	14.5	1,018
Erythromycin	Serum	0.3 ± 0.1	1	0.8	1
	Lung	2.9 ± 0.02	2	0.8	1

[a] Values are calculated from mean concentrations in serum and lung tissue taken at 0.5, 1, 2, 4, 8, 24, 48, 72, and 96 h postdosing.
[b] Values are means ± standard deviations for three samples.

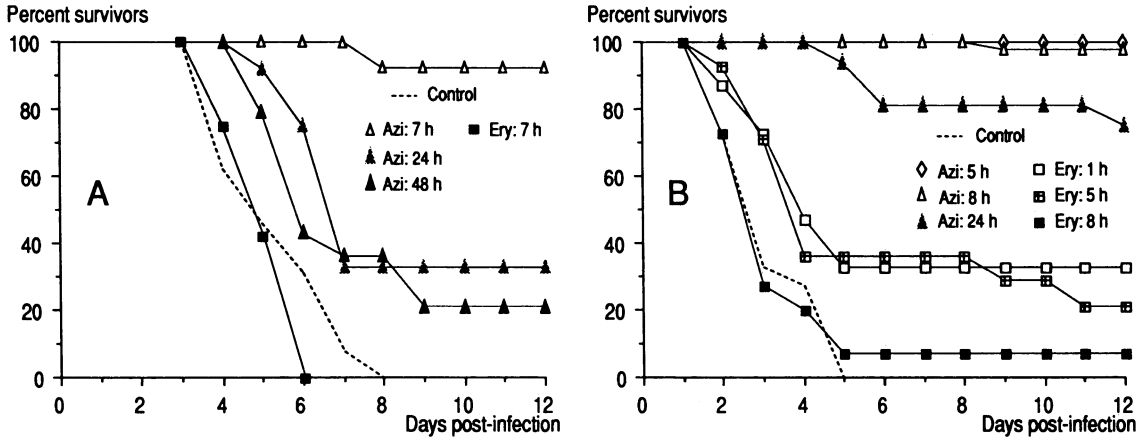

FIG. 1. Effects of prophylactic treatment with azithromycin (Azi) or erythromycin (Ery) on survival rates in *S. pneumoniae*-infected mice. (A) Subacute pneumonia model (C57BL/6 mice). Mice received a single p.o. dose of 25 mg/kg at 7, 24, or 48 h before bacterial challenge (azithromycin) or 7 h before bacterial challenge (erythromycin). (B) Acute pneumonia model (Swiss mice). Mice received a single s.c. dose of 50 mg/kg at 5, 8, or 24 h before infection for azithromycin treatment and at 1, 5, or 8 h for erythromycin treatment.

The elimination of erythromycin from serum was much more rapid, with a $t_{1/2}$ of 0.8 h. Both azithromycin and erythromycin displayed a short lag in absorption in terms of the T_{max}, with erythromycin attaining peak concentrations earlier than azithromycin. The serum AUC of azithromycin was 2.7-fold higher than that of erythromycin. The lung distribution profiles showed that azithromycin achieved a peak level in tissue that was 2.2-fold higher than that of erythromycin. The residence time of azithromycin in the lung was much longer than that of erythromycin (96 versus <8 h). The azithromycin elimination $t_{1/2}$ in the lung was 17 h, i.e., as much as 21 times longer than that of erythromycin. Erythromycin was rapidly eliminated from the lung, in parallel with its curve of elimination from serum. The differences in the tissue pharmacokinetics between azithromycin and erythromycin were most striking in terms of AUC, with azithromycin values being 19-fold higher than erythromycin values. The distribution in the lungs, expressed as the ratio of tissue AUC/serum AUC, was much greater for azithromycin than for erythromycin (21 versus 3). A similar pharmacokinetic pattern was found for both drugs in infected mice, with the trend for azithromycin being toward higher lung diffusion than that in noninfected controls (Table 1).

When the antibiotics were administered p.o. (Table 2) to healthy controls, pharmacokinetic profiles paralleled those following s.c. administration. Azithromycin again had a much longer $t_{1/2}$ in both serum and lung (4.4 and 17.9 h, respectively) than erythromycin (0.7 and 1.1 h, respectively), and the azithromycin AUCs (20 and 697 μg · h/ml or μg · h/g in serum and lung, respectively) greatly exceeded those of erythromycin (8 and 39 μg · h/ml or μg · h/g, respectively). In infected animals, levels of azithromycin in sera and lungs were the same as those in noninfected controls, but those of erythromycin were very much decreased. Moreover, the respective serum and lung AUCs for erythromycin were both 1 μg · h/ml compared with 23 and 1,018 μg · h/ml for azithromycin.

Protection studies. (i) Prophylactic regimens. In the model of subacute infection (Fig. 1A), a single p.o. dose of 25 mg/kg azithromycin given 7 h prior to challenge protected 92% of the mice, while erythromycin administered at the same dose was completely ineffective. Moreover, 20% of the mice survived when azithromycin was given 48 h before the infection. In the model of acute infection (Fig. 1B), when azithromycin was administered as a single s.c. 50-mg/kg dose at 8 or 24 h before infection, 100 and 80% of the mice survived, respectively. The same dose of erythromycin protected only 7% of the animals when it was given 8 h before infection; survival increased to only 35% when erythromycin was given 1 h before the challenge.

(ii) Therapeutic regimens. Therapy was initiated 48 h postinfection, a time corresponding in the model of subacute infection to high bacterial growth and an inflammatory response in the lung. Two consecutive doses of 12.5 mg of azithromycin per kg given p.o. gave 80% survival, compared with 7% survival with the same erythromycin treatment (Fig. 2A). A single s.c. injection of azithromycin, 50 mg/kg, protected 95% of the mice, whereas two to four injections of erythromycin at the same dose were required to reach similar (80 to 87%) survival rates (Fig. 2B).

Pulmonary clearance. Lung clearance data (Table 3) correlated with the survival rates. The total *S. pneumoniae* CFU recovered from the lungs at 65 h postchallenge, 17 h after a single injection of azithromycin, 25 mg/kg, was significantly lower than that from the lungs of untreated controls. At that time, the bacterial populations recovered from the lungs of mice treated with azithromycin were also significantly lower than those recovered from the lungs of animals treated with erythromycin. Twenty-four hours after a second antibiotic injection, bacteria were undetectable in the lungs of azithromycin-treated mice, whereas bacterial counts for erythromycin-treated animals were only 30% lower than those for controls. After two injections of erythromycin, clearance of bacteria was significantly greater than that in controls, but bacterial regrowth was detected 6 days after the second injection. Bacteria were always found in the blood of controls and erythromycin-treated mice, while blood from azithromycin-treated mice was sterile 24 h after the second injection.

DISCUSSION

Our data show that azithromycin has greater efficacy than erythromycin against *S. pneumoniae* in both acute (Swiss mice) and subacute (C57BL/6 mice) pneumonia, whether given prophylactically or therapeutically. A single dose of

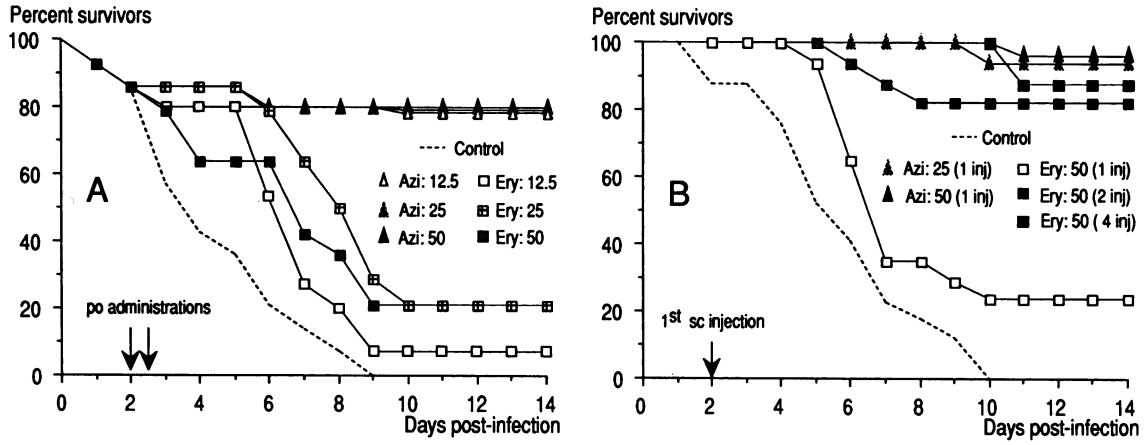

FIG. 2. Effects of curative treatment with azithromycin (Azi) or erythromycin (Ery) on survival rates in *S. pneumoniae*-infected C57BL/6 mice (model of subacute infection). (A) p.o. route. Treatment was given in two doses of 12.5, 25, or 50 mg/kg for both drugs. (B) s.c. route. Treatment was given in a single dose of 25 or 50 mg/kg for azithromycin and in one, two, or four injections of 50 mg/kg for erythromycin.

azithromycin (50 mg/kg, s.c.) administered 24 h before infection protected 80% of the mice. In contrast, even when given very close to the challenge (1 h before), erythromycin protected only 35% of the animals. These findings are in general agreement with those of Retsema et al. (10). Azithromycin has also been reported to be more effective than erythromycin against a wide range of infections in animal models (4). With regard to therapy, a single dose of azithromycin (25 mg/kg, s.c.) at 48 h postinfection was protective, whereas two to four injections of erythromycin (50 mg/kg at 12-h intervals) were required to reach a similar survival rate. Bacterial clearance data closely paralleled survival. A single 25-mg/kg injection of azithromycin at 48 h postinfection reduced lung bacterial counts to a very low level. In contrast, two injections of erythromycin at the same dose did not prevent bacterial regrowth. Retsema et al. (11) also found that treatment of mice with azithromycin at 25 mg/kg significantly reduced the number of *S. pneumoniae* recovered from the lungs, whereas after treatment with erythromycin at 100 mg/kg, counts were similar to those in untreated controls.

The in vitro activity of azithromycin does not account for its higher in vivo efficacy. The MIC and MBC of azithromycin against the *S. pneumoniae* strain used here were 0.125 and 0.25 μg/ml, respectively, i.e., twofold higher than those for erythromycin (0.06 and 0.12 μg/ml, respectively). However, pharmacokinetic findings in both noninfected and infected mice showed that after a single s.c. or p.o. dose of 50 mg/kg, the concentrations of azithromycin exceeded the MBC for the pathogen for 24 and 96 h in sera and lungs, respectively. At the same dosage, erythromycin levels exceeded the MBC for *S. pneumoniae* for less than 8 h in both sera and lungs.

Azithromycin showed continuously high levels in the sera and lungs of noninfected controls, regardless of the route of administration. Similarly, azithromycin given s.c. or p.o. had much longer serum and lung $t_{1/2}$s in healthy controls than those of erythromycin, and the AUCs in serum and lung exceeded those of erythromycin by a large margin. In infected animals, azithromycin maintained its superior pharmacokinetic profile relative to that of erythromycin; this was most striking following p.o. administration, since erythromycin lost all activity. The stability of azithromycin in the acidic medium of the stomach, as well as in the inflammatory sites of infection, contribute to its excellent pharmacokinetic profile. Fiese and Steffen (2) have shown that in acidic aqueous media, erythromycin is rapidly degraded to products that possess little antimicrobial activity. Azithromycin has a methyl-substituted nitrogen in place of the carbonyl at the 9a position of the aglycone ring, blocking the internal dehydration pathway. In solution at 37°C and pH 2, 10% of azithromycin is degraded after 20 min, compared with only

TABLE 3. Bacterial clearance of *S. pneumoniae* from lung and blood of C57BL/6j mice treated with s.c. injections of antibiotic at 25 mg/kg initiated 48 h after infection

Time[a]	Control		Azithromycin		Erythromycin	
	Lung ($\log_{10}$ CFU/ml)[b]	Blood (no. of positive cultures/ total no.)	Lung ($\log_{10}$ CFU/ml)[b]	Blood (no. of positive cultures/ total no.)	Lung ($\log_{10}$ CFU/ml)[b]	Blood (no. of positive cultures/ total no.)
H + 65 (17 h after single injection)	7.52 ± 0.44	3/3	2.98 ± 0.54[c,d]	3/3	6.33 ± 1.29	3/3
H + 89 (24 h after second injection)	7.55 ± 0.55	3/3	<2.6[c,d,e]	0/3	5.27 ± 0.56[f]	3/3
H + 233 (6 days after second injection)	7.84 ± 0.02	3/3	<2.6[c,d,e]	0/3	7.80 ± 0.03	3/3

[a] H indicates time after infection.
[b] Values are means ± standard deviations (n = 3) of viable bacteria in lungs ($\log_{10}$ CFU per milliliter of lung homogenate).
[c] $P < 0.001$ versus control.
[d] $P < 0.001$ versus erythromycin.
[e] The lower limit of detection was 2.6 $\log_{10}$ units of CFU/ml (4×10^2 CFU/ml).
[f] $P < 0.05$ versus control.

3.7 s for erythromycin. The greater acid stability of azithromycin may account for the higher concentrations in plasma reached following oral administration than those obtained with erythromycin. Azithromycin given p.o. has also been shown to be well absorbed in animals (4, 12) and humans (3). Furthermore, azithromycin is distributed within the tissues more rapidly and at higher concentrations than erythromycin. While most studies have concerned healthy hosts, our results show that the pharmacological advantages of azithromycin are maintained and possibly enhanced in infected mice. The resulting higher and sustained levels of azithromycin in tissue could explain the better efficacy of azithromycin relative to that of erythromycin in our *S. pneumoniae* pneumonia model. The in vivo performance of azithromycin may also be related to its intracellular concentration and release at sites of infection (5), which would also explain the efficacy of azithromycin against intracellular pathogens.

In conclusion, our results show that azithromycin, a new azalide antibiotic, is highly effective in an experimental model of pneumonia induced by a macrolide-susceptible strain of *S. pneumoniae*. Clinical evaluation of this antibiotic in the treatment of patients with community-acquired pneumonia appears to be justified since azithromycin demonstrates excellent in vitro and in vivo activities not only against *S. pneumoniae* but also against gram-negative bacteria, including *H. influenzae* (9–11) and the family of intra- and extracellular bacterial pathogens involved in respiratory tract infections.

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