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In Vitro and In Vivo Activities of Macrolides against *Mycoplasma pneumoniae*

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We investigated the in vitro and in vivo activities of macrolides against *Mycoplasma pneumoniae*. In vitro MICs of azithromycin, erythromycin, clarithromycin, and roxithromycin were determined. Azithromycin was the most potent antimicrobial agent tested in vitro. Its MIC for 90% of the strains was 0.00024 μg/ml. MICs for 90% of the strains of erythromycin, clarithromycin, and roxithromycin were 0.0156, 0.0078, and 0.03125 μg/ml, respectively. In vivo activities were assessed in a pulmonary infection model with Syrian golden hamsters. We evaluated the in vivo effects on reduction of viable *M. pneumoniae* cell counts and on reduction of microscopic and macroscopic histopathologies for azithromycin, erythromycin, and clarithromycin given at 10 mg/kg once daily for 1 and 3 days and given at 15 mg/kg twice daily for 2.5 and 5 days. Azithromycin was significantly more effective than erythromycin or clarithromycin in the same regimens. Especially at 10 mg/kg once daily for 1 day, only azithromycin was significantly effective in the reduction of viable *M. pneumoniae* cells and histopathologies. These results show that azithromycin is more efficacious than the other drugs tested against *M. pneumoniae* pneumonia in hamsters. These data suggest that clinical studies of macrolides in human patients are warranted.

Mycoplasma pneumoniae is a major cause of pneumonia and accounts for as many as 20% of the total number of cases of pneumonia (6). The recommended therapy is erythromycin, which is efficacious in reducing the duration of symptoms (19). Recently, it has been suggested that new macrolides (8, 10), such as azithromycin, clarithromycin, and roxithromycin, may be used more frequently for respiratory tract infections because of their improved potencies and pharmacokinetic parameters compared with those of erythromycin (10). Azithromycin is a new azalide antibiotic (7, 15) that differs from erythromycin by a methyl-substituted nitrogen at position 9a within the macrocyclic ring. This modification has resulted in improved potency against gram-negative bacteria. Moreover, azithromycin proved to be superior to erythromycin in a mouse pneumococcal model because of these improved pharmacokinetic parameters (1).

In this study we evaluated the in vitro antimycoplasmal activities of azithromycin, erythromycin, clarithromycin, and roxithromycin. We also tested the in vivo antimycoplasmal efficacies of azithromycin, erythromycin, and clarithromycin in courses of 10 mg/kg once daily for 1 or 3 days or 15 mg/kg twice daily for 2.5 or 5 days by using an experimental pulmonary infection model with Syrian golden hamsters (2).

MATERIALS AND METHODS

Compounds. Azithromycin was kindly supplied by Pfizer Inc. Other antimicrobial agents, i.e., erythromycin (Dainippon Pharmaceutical Co. Ltd.), clarithromycin (Taishiyo Pharmaceutical Co. Ltd.), and roxithromycin (Roussel Pharmaceutical Co. Ltd.), were supplied by the indicated pharmaceutical companies. Macrolides were dissolved in ethanol and then diluted in modified Chanock broth medium A for determination of MICs. Each drug was dissolved or suspended in 5% arabic gum for oral administration.

Organisms. Forty clinically isolated strains of *M. pneumoniae* obtained from 1988 to 1992 at Nagasaki University Hospital and three standard strains, FH, Mac, and M129, which were supplied by M. F. Barile (Food and Drug Administration, Bethesda, Md.), were used for in vitro tests, and the virulent strain M129 at passage level 12 was used for experimental mycoplasmal pneumonia in Syrian golden hamsters.

MIC determinations. MICs were determined by using a modified broth microdilution method (12, 22, 23) with modified Chanock broth medium (3), which consisted of seven parts PPLO broth without crystal violet (Difco Laboratories, Detroit, Mich.), 2 parts uninactivated horse serum, 1 part 25% fresh yeast extract, 1% glucose, and 0.002% phenol red adjusted to pH 7.8 with 1 N sodium hydroxide. Inocula were 10^5 CFU/ml. The plates, sealed with plate sealers, were incubated at 37°C in air. In each case, when the color of the medium of the drug-free control changed from red to yellow, the minimal concentration of drug preventing the color change was defined as the MIC. As a control for potential interactions between antibiotics, medium components, and pH, which could potentially affect the observed MICs, the American Type Culture Collection bacterial reference strain *Staphylococcus aureus* ATCC 29213 (for which MICs of the drugs tested, obtained with Mueller-Hinton broth, have been published [24]) was inoculated into microtiter plates containing Chanock broth. A positive (growth) control consisting of organisms in broth, a negative (sterility) control consisting of uninoculated broth, and a drug control consisting of broth containing the highest concentration of drug tested were included for each *M. pneumoniae* strain tested.

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TABLE 1. Microscopic parameters for evaluating severity of mycoplasmal pneumonia in hamster lungs

Score[a]	No. of sites[b] affected	Peribronchial and peribronchiolar infiltrates	Lumenal exudate
0	None	None	None
1	Few	Mild	Mild
2	Many	Moderate	Moderate
3	Most	Severe	Severe
4	All		

[a] The microscopic pathology score was defined as the sum of the scores for number of sites affected, peribronchial and peribronchiolar infiltrates, and lumenal exudate.

[b] Site refers to any peribronchial and peribronchiolar infiltrates.

Infection of hamsters. In vivo activities of macrolides were assessed with a pulmonary infection model using Syrian golden hamsters (2, 20). Each dosing group consisted of 10 6-week-old male Syrian golden hamsters weighing about 100 g (Japan SLC, Shizuoka, Japan). Five hamsters in each dosing group were sacrificed and submitted for study of viable mycoplasma cells, and the remainder were used for the microscopic pathology study. The lungs of all hamsters were also examined macroscopically when their thoraxes were surgically opened. Hamsters were anesthetized by intraperitoneal injection of ketamine (Sankyo Co., Ltd.) and infected by a peroral intratracheal intubation method described previously (2), with modifications. The hamsters were mounted upside down on a 45° angle from vertical. A bent, blunted 2-in. (ca. 5-cm) 20-gauge Angiocath (Becton Dickinson Vascular Access) was inserted under the glottis into the trachea, and the inner needle was drawn. A 1-ml tuberculin syringe was attached to the outer catheter and moved as far forward as possible. A 0.15-ml aliquot of the organism suspension containing 7.5×10^6 CFU of *M. pneumoniae* M129 at passage level 12 was inoculated directly into the trachea. Control intratracheal inoculations with India ink verified the limited distribution of the inoculum into the right lung, especially the lower right lobe. For a negative control, broth not containing *M. pneumoniae* was also inoculated, and no lung lesions were observed microscopically or macroscopically after that inoculation.

Therapeutic plan. The in vivo activities of macrolides against experimentally induced *M. pneumoniae* pneumonia in hamsters were examined by tube feeding. Oral administration of azithromycin, erythromycin, and clarithromycin (each given at 10 mg/kg once daily for 1 and 3 days and at 15 mg/kg twice daily for 2.5 and 5 days) was started on day 5 after inoculation of *M. pneumoniae.*

In groups treated with 10 mg/kg once daily for 1 day, hamsters were sacrificed on days 7, 9, and 11 after infection. In groups treated with 10 mg/kg once daily for 3 days, hamsters were sacrificed on days 9 and 11 after infection. In groups treated with 15 mg/kg twice daily for 2.5 days, hamsters were sacrificed on day 9 after infection. In groups treated with 15 mg/kg twice daily for 5 days, hamsters were sacrificed on day 11 after infection. For the untreated control, oral administration of 5% arabic gum solution was started on day 5 after inoculation of *M. pneumoniae* and continued for 1 day once only and for 2.5 and 5 days twice daily. After 2 days for each untreated control, 10 hamsters in each case were sacrificed and their lungs were examined. Before treatment (on day 5 after infection), measurement of the number of viable mycoplasma cells in lungs and estimation of severity of histopathologies were also done.

Pulmonary clearance studies. The lungs were removed aseptically, weighed, and homogenized in 5 ml of modified Chanock broth (consisting of 7 parts PPLO broth without crystal violet, 2 parts uninactivated horse serum, 1 part 25% fresh yeast extract, 1% glucose, 0.002% phenol red, 1,000 U of penicillin G per ml, and 0.025% thallium acetate) by using a glass homogenizer. After homogenization, each mixture was made up to 10 ml by the addition of modified Chanock broth medium to the homogenized suspensions. Serial 10-fold dilutions of the homogenized suspensions with modified Chanock broth were made, from 10^0 to 10^{-8}. Twenty 0.01-ml portions (total, 0.2 ml) of each dilution were spotted on five Chanock agar plates (four spots on each agar plate) consisting of 7 parts PPLO agar, 2 parts uninactivated horse serum, 1 part 25% fresh yeast extract, 1,000 U of penicillin G per ml, and 0.025% thallium acetate. Plates were incubated aerobically at 37°C for 14 days in a tightly closed vinyl bag to maintain humidity, and the number of CFU per gram of lung was calculated from the number of colonies developed on the five plates used for each dilution (17). *M. pneumoniae* was not recovered from the plates with the 10^0 and 10^{-1} dilutions after azithromycin therapy because of the high concentration of azithromycin remaining in the medium, so colony counts for all therapeutic courses were determined with plates with dilutions of 10^{-2} or 10^{-3}. Some isolated colonies were identified by inhibition of growth in the presence of anti-*M. pneumoniae* rabbit serum and by their hemadsorption properties (4). For the untreated control, oral administration of 5% arabic gum solution was started on day 5 after inoculation of *M. pneumoniae* and continued for 1 day once only and for 2.5 and 5 days twice daily. After 2 days for each untreated control, five hamsters in each case were sacrificed and their lungs were quantitatively cultured. Before treatment (day 5 after infection), measurement of the number of viable mycoplasma cells in lungs was also done.

Assessment of lung lesions. Counting of viable mycoplasmal cells after therapy was not sufficient for estimating the therapeutic effects of the drugs, so we also tried to estimate the severity of pneumonia microscopically and macroscopically.

(i) Microscopic lung lesions. Five hamsters in each group were sacrificed, and their lungs were fixed by in situ intratra-

TABLE 2. Susceptibilities of 40 *M. pneumoniae* clinical strains and standard strains M129, Mac, and FH to azithromycin, clarithromycin, roxithromycin, and erythromycin

Drug	MIC (μg/ml)					
	Range	For 50% of strains	For 90% of strains	M129	Mac	FH
Azithromycin	0.00006–0.00024	0.00012	0.00024	0.00012	0.00024	0.00024
Erythromycin	0.0039–0.03125	0.0078	0.0156	0.0156	0.0156	0.0076
Clarithromycin	0.00195–0.0156	0.0039	0.0078	0.0156	0.0156	0.0076
Roxithromycin	0.0039–0.03125	0.0156	0.03125	0.03125	0.03125	0.03125

cheal instillation of 10% phosphate-buffered formalin under controlled pressure. Thin sections of lung (in paraffin) were stained with hematoxylin and eosin. The severity of lung lesions was scored microscopically by estimating the degree of peribronchial and peribronchiolar infiltrates and lumenal exudates and by estimating the numbers of sites affected in three separate coronal sections including the main bronchus of the right lower lobe, to which the lung lesions were limited, using the criteria in Table 1 (see Fig. 3). These criteria were established by modifying the criteria described previously by Barile et al. (2). This trial was performed blindly by two investigators, and the average score was recorded.

(ii) Macroscopic lung lesions. Lesions in the lung were limited to the right lower lobe, so the severity of pneumonia could be estimated macroscopically by measuring the extent of macroscopic lung lesions in the right lower lobe. Small differences in the extents of macroscopic lung lesions were difficult to distinguish, so the severity of pneumonia was classified into six groups according to the extent of macroscopic lesions, and a ratio representing each group was determined, as follows (see Fig. 6): lesion less than one-eighth of the lobe, 0; more than one-eighth and less than one-fourth, 1/8; more than one-fourth and less than one-half, 1/4; more than one-half and less than three-fourths, 1/2; more than three-fourths and less than one, 3/4; and more than one lobe, 1. This trial was also done blindly.

Levels of azithromycin, erythromycin, and clarithromycin in lungs. Concentrations of azithromycin, erythromycin, and clarithromycin in lungs taken from uninfected 6-week-old male Syrian golden hamsters weighing about 100 g were determined. Samples were taken 0.5, 1, 2, 4, 6, 12, and 24 h following administration of a single oral dose of 50 mg/kg. Three hamsters were sacrificed by cutting the abdominal aorta at each time point, and lungs were removed. Lungs were weighed and homogenized in 0.067 M phosphate buffer (pH 7.4). After centrifugation, supernatants were obtained. Samples were assayed for bioactivity by an agar well diffusion procedure with *Micrococcus luteus* ATCC 9341 as the test organism by the method described previously by Girard et al. (7).

Statistical analysis. Differences in CFU, microscopic scores, and ratios of macroscopic lung lesions between the treated and untreated groups were evaluated by use of the Mann-Whitney U test for nonparametric analysis. All analyses were conducted with Version II Statview SE software (Abacus Concepts, Inc.).

RESULTS

In vitro activities. The in vitro antimycoplasmal activities of the macrolides tested are shown in Table 2. The MICs of the macrolides against *S. aureus* ATCC 29213 in Chanock broth were around one-fourth (or less) of those against *S. aureus* ATCC 29213 in Mueller-Hinton broth on day 1, when the growth of the positive control was confirmed (Table 3). This reflected the fact that horse serum increases the potency of macrolide antibiotics, as had been generally known. With this consideration, our determinations of MICs against *M. pneumoniae* and *S. aureus* ATCC 29213 in Chanock broth were considered to be acceptable.

In vivo activities. Data on the effectiveness of each regimen of azithromycin, erythromycin, and clarithromycin in reducing viable *M. pneumoniae* cell counts and microscopic and macroscopic histopathologies are collected in Table 4.

Viable mycoplasma cell count. The numbers of viable mycoplasma cells in the lungs after oral administration following experimentally induced pneumonia in Syrian golden hamsters are shown in Fig. 1.

TABLE 3. MICs of azithromycin, erythromycin, clarithromycin, and roxithromycin for *S. aureus* ATCC 29213 in different media

Drug	MIC (μg/ml)					
	Mueller-Hinton broth (pH 7.4)			Chanock broth (pH 7.8)		
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5
Azithromycin	1	1	1	0.25	1	2
Erythromycin	0.5	1	1	0.06	0.5	0.5
Clarithromycin	0.25	0.5	1	0.03	0.25	0.5
Roxithromycin	0.5	1	1	0.125	0.5	1

On day 7 after infection, compared with the untreated control, azithromycin at 10 mg/kg once daily for 1 day was effective ($P < 0.01$), whereas erythromycin and clarithromycin once daily for 1 day were not effective.

On day 9 after infection, compared with the untreated control, azithromycin at 10 mg/kg once daily for 1 day, at 10 mg/kg once daily for 3 days, and at 15 mg/kg twice daily for 2.5 days was effective ($P < 0.01$), and erythromycin at 15 mg/kg twice daily for 2.5 days ($P < 0.01$) and clarithromycin at 10 mg/kg once daily for 3 days and at 15 mg/kg twice daily for 2.5 days were also effective ($P < 0.01$), whereas erythromycin at 10 mg/kg once daily for 1 day and at 10 mg/kg once daily for 3 days and clarithromycin at 10 mg/kg once daily for 1 day were not effective. On day 9 after infection, azithromycin at 10 mg/kg once daily for 1 day, 10 mg/kg once daily for 3 days, and 15 mg/kg twice daily for 2.5 days was more effective than the corresponding regimens of erythromycin and clarithromycin ($P < 0.05$).

On day 11 after infection, compared with the untreated control, azithromycin at 10 mg/kg once daily for 1 day and at 10 mg/kg once daily for 3 days was effective ($P < 0.01$), and erythromycin at 15 mg/kg twice daily for 5 days ($P < 0.01$) and clarithromycin at 10 mg/kg once daily for 3 days ($P < 0.05$) and at 15 mg/kg twice daily for 5 days ($P < 0.01$) were effective. No recovery of *M. pneumoniae* was observed after administration of azithromycin at 15 mg/kg twice daily for 5 days, but because a high concentration of azithromycin, which prevents *M. pneumoniae* from growing, may remain in the medium as judged by the results of studying azithromycin levels in the lung, it seems that the effect of azithromycin at 15 mg/kg twice daily for 5 days should not be evaluated. On day 11 after infection, azithromycin at 10 mg/kg once daily for 1 day and at 10 mg/kg once daily for 3 days was more effective than the corresponding regimens of erythromycin and clarithromycin ($P < 0.01$). There was a statistically significant difference between results with the early control (day 5 after infection) and the untreated control on day 7. There was also a statistically significant difference between results with the untreated control on day 7 after infection and the untreated control on day 9 and between results with the untreated control on day 9 and the untreated control on day 11.

Histological evaluations. Data relating to microscopic pathological scores and area ratios of macroscopic lung lesions in the right lower lobe after oral administration of drugs following experimentally induced pneumonia in Syrian golden hamsters are shown in Fig. 2 to 5. A macroscopic lung lesion (Fig. 6) is shown to be composed primarily of cell infiltrations into interalveolar septums when it is observed microscopically (Fig. 2). Thus, microscopic study was useful mainly for estimating the severity of peribronchial and peribronchiolar lesions, and macroscopic study was useful mainly for estimating the severity of peripheral lesions, which include the interalveo-

TABLE 4. Effectiveness of various regimens of azithromycin, erythromycin, and clarithromycin in reduction of viable *M. pneumoniae* cell counts and microscopic and macroscopic histopathologies[a]

Regimen and drug	Effectiveness								
	Day 7			Day 9			Day 11		
	Cell	Microscopic	Macroscopic	Cell	Microscopic	Macroscopic	Cell	Microscopic	Macroscopic
10 mg/kg once daily for 1 day									
AZM	E	E	E	E	E	E	E	E	E
EM	NE	NE	NE	NE	NE	NE	NE	NE	NE
CAM	NE	NE	NE	NE	E	E	NE	E	E
10 mg/kg once daily for 3 days									
AZM				E	E	E	E	E	E
EM				NE	E	E	NE	NE	E
CAM				E	E	E	E	E	E
15 mg/kg twice daily for 2.5 days									
AZM				E	E	E			
EM				E	E	E			
CAM				E	E	E			
15 mg/kg twice daily for 5 days									
AZM							—	E	E
EM							E	E	E
CAM							E	E	E

[a] Abbreviations: AZM, azithromycin; EM, erythromycin; CAM, clarithromycin; E, effective statistically compared with untreated control; NE, not effective statistically compared with untreated control; —, unable to be estimated; cell, reduction of viable *M. pneumoniae* cell count; microscopic, reduction of microscopic histopathologies; macroscopic, reduction of macroscopic histopathologies.

lar septum and lung alveolus. In microscopic and macroscopic estimations there was no statistically significant difference between the early control (day 5 after infection) and the untreated control on days 7, 9, and 11, which showed that there was no spontaneous microscopic or macroscopic histologic reduction in mycoplasmal pneumonia in the hamsters until day 11 after infection. This result shows that the natural histopathological course of mycoplasmal pneumonia in hamsters was not parallel with the natural course of viable *M. pneumoniae* numbers in the hamsters' lungs.

Microscopic histopathology estimations. On day 7 after infection, compared with the untreated control, azithromycin at 10 mg/kg once daily for 1 day was effective for reducing microscopic histopathological severity ($P < 0.05$), whereas erythromycin and clarithromycin were not effective.

On day 9 after infection, compared with the untreated

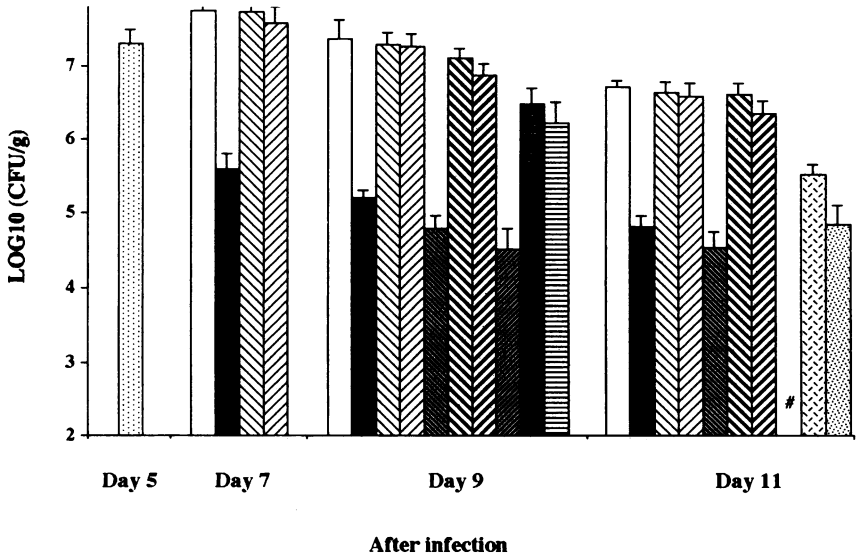

FIG. 1. Numbers of viable *M. pneumoniae* cells during experimentally induced *M. pneumoniae* pneumonia in hamsters. Results are means for five hamsters per group with standard deviations. Column numbers (except as noted), counting from left to right: 1, early control; 2, untreated control; 3, azithromycin at 10 mg/kg once daily for 1 day; 4, erythromycin at 10 mg/kg once daily for 1 day; 5, clarithromycin at 10 mg/kg once daily for 1 day; 10, azithromycin at 10 mg/kg once daily for 3 days; 11, erythromycin at 10 mg/kg once daily for 3 days; 12, clarithromycin at 10 mg/kg once daily for 3 days; 13, azithromycin at 15 mg/kg twice daily for 2.5 days; 14, erythromycin at 15 mg/kg twice daily for 2.5 days; 15, clarithromycin at 15 mg/kg twice daily for 2.5 days; #, no recovery of *M. pneumoniae* observed after azithromycin at 15 mg/kg twice daily for 5 days; 23, erythromycin at 15 mg/kg twice daily for 5 days; 24, clarithromycin at 15 mg/kg twice daily for 5 days.

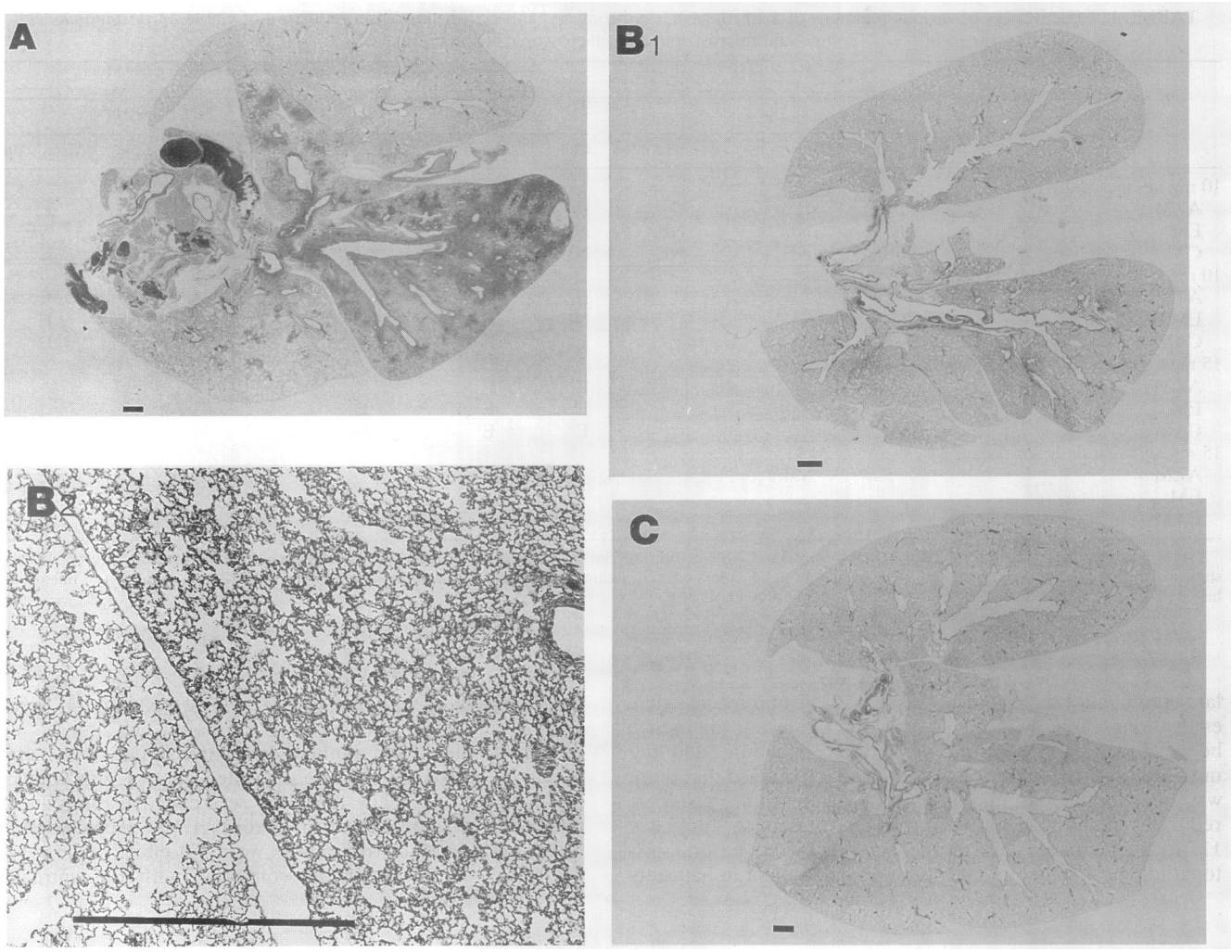

FIG. 2. Hamster lungs on day 11 after infection with *M. pneumoniae*. Bars, 1 mm. (A) Untreated control. Severe cell infiltration into the lung alveolus and interalveolar septum and severe peribronchial and peribronchiolar infiltrates are observed in the whole right lower lobe. Magnification, ×3. (B_1) Treatment with azithromycin at 10 mg/kg once daily for 3 days. Mild cell infiltration into the lung alveolus and interalveolar septum and mild peribronchial and peribronchiolar infiltrates are observed in the right lower lobe. The lesion in right lower lobe is definitely reduced. Magnification, ×3. (B_2) Magnification (×40) of panel B_1. Cell infiltrations into the interalveolar septum are observed in the area corresponding to macroscopic lung consolidation. (C) Normal hamster lung. No cell infiltration into the lung alveolus and interalveolar septum and no peribronchial and peribronchiolar infiltrates are observed. Magnification, ×3.

control, azithromycin at 10 mg/kg once daily for 1 day ($P < 0.01$), at 10 mg/kg once daily for 3 days ($P < 0.01$), and at 15 mg/kg twice daily for 2.5 days ($P < 0.01$); erythromycin at 10 mg/kg once daily for 3 days ($P < 0.05$) and at 15 mg/kg twice daily for 2.5 days ($P < 0.05$); and clarithromycin at 10 mg/kg once daily for 1 day ($P < 0.05$), at 10 mg/kg once daily for 3 days ($P < 0.01$), and at 15 mg/kg twice daily for 2.5 days ($P < 0.01$) were effective. On day 9 after infection, azithromycin at 10 mg/kg once daily for 1 day ($P < 0.01$), at 10 mg/kg once daily for 3 days ($P < 0.01$), and at 15 mg/kg twice daily for 2.5 days ($P < 0.01$) was more effective than the corresponding regimens of erythromycin and clarithromycin. There were no significant differences among results for azithromycin at 10 mg/kg once daily for 1 day, 10 mg/kg once daily for 3 days, and 15 mg/kg twice daily for 2.5 days on day 9.

On day 11 after infection, compared with the untreated control, azithromycin at 10 mg/kg once daily for 1 day ($P < 0.01$), at 10 mg/kg once daily for 3 days ($P < 0.01$), and at 15 mg/kg twice daily for 5 days ($P < 0.01$); erythromycin at 15 mg/kg twice daily for 5 days ($P < 0.05$); and clarithromycin at 10 mg/kg once daily for 1 and 3 days ($P < 0.05$) and at 15 mg/kg twice daily for 5 days ($P < 0.05$) were effective. On day 11 after infection, azithromycin at 10 mg/kg once daily for 1 day ($P < 0.01$), 10 mg/kg once daily for 3 days ($P < 0.01$), and 15 mg/kg twice daily for 5 days ($P < 0.05$) was more effective than the corresponding regimens of erythromycin and clarithromycin. There were no significant differences among results for azithromycin at 10 mg/kg once daily for 1 day, 10 mg/kg once daily for 3 days, and 15 mg/kg twice daily for 5 days on day 11.

Macroscopic histopathology estimations. On day 7 after infection, compared with the untreated control, azithromycin was effective ($P < 0.01$), whereas erythromycin and clarithromycin at 10 mg/kg once daily for 1 day were not effective for reducing macroscopic histopathological severity. On day 9 after infection, compared with the untreated control, azithromycin at 10 mg/kg once daily for 1 day ($P < 0.05$), at 10 mg/kg

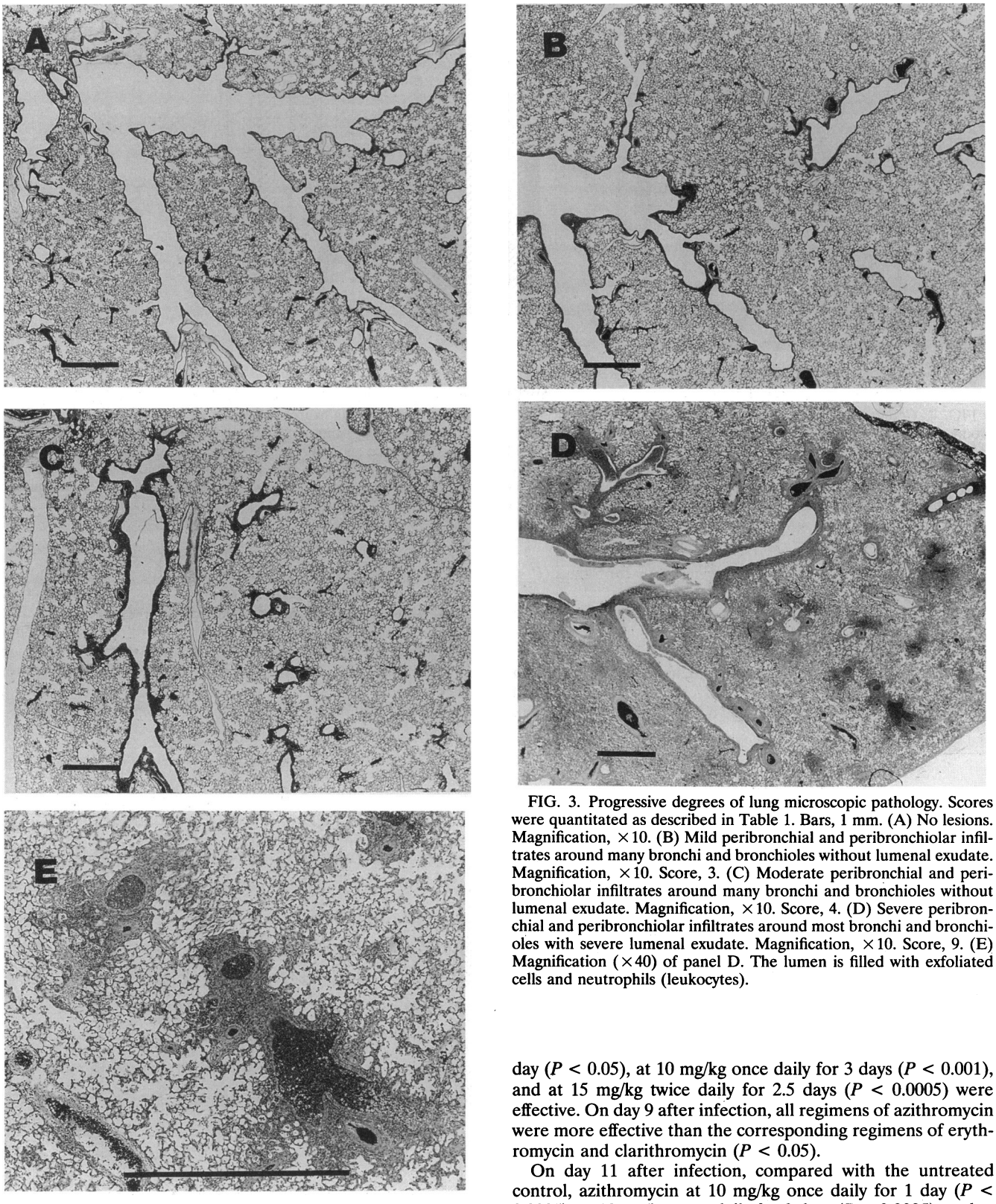

FIG. 3. Progressive degrees of lung microscopic pathology. Scores were quantitated as described in Table 1. Bars, 1 mm. (A) No lesions. Magnification, ×10. (B) Mild peribronchial and peribronchiolar infiltrates around many bronchi and bronchioles without lumenal exudate. Magnification, ×10. Score, 3. (C) Moderate peribronchial and peribronchiolar infiltrates around many bronchi and bronchioles without lumenal exudate. Magnification, ×10. Score, 4. (D) Severe peribronchial and peribronchiolar infiltrates around most bronchi and bronchioles with severe lumenal exudate. Magnification, ×10. Score, 9. (E) Magnification (×40) of panel D. The lumen is filled with exfoliated cells and neutrophils (leukocytes).

once daily for 3 days ($P < 0.001$), and at 15 mg/kg twice daily for 2.5 days ($P < 0.001$); erythromycin at 10 mg/kg once daily for 3 days ($P < 0.05$) and at 15 mg/kg twice daily for 2.5 days ($P < 0.001$); and clarithromycin at 10 mg/kg once daily for 1 day ($P < 0.05$), at 10 mg/kg once daily for 3 days ($P < 0.001$), and at 15 mg/kg twice daily for 2.5 days ($P < 0.0005$) were effective. On day 9 after infection, all regimens of azithromycin were more effective than the corresponding regimens of erythromycin and clarithromycin ($P < 0.05$).

On day 11 after infection, compared with the untreated control, azithromycin at 10 mg/kg once daily for 1 day ($P < 0.0005$), at 10 mg/kg once daily for 3 days ($P < 0.0005$), and at 15 mg/kg twice daily for 5 days ($P < 0.0005$); erythromycin at 10 mg/kg once daily for 3 days ($P < 0.01$) and at 15 mg/kg twice daily for 5 days ($P < 0.01$); and clarithromycin at 10 mg/kg once daily for 1 day ($P < 0.05$), at 10 mg/kg once daily for 3

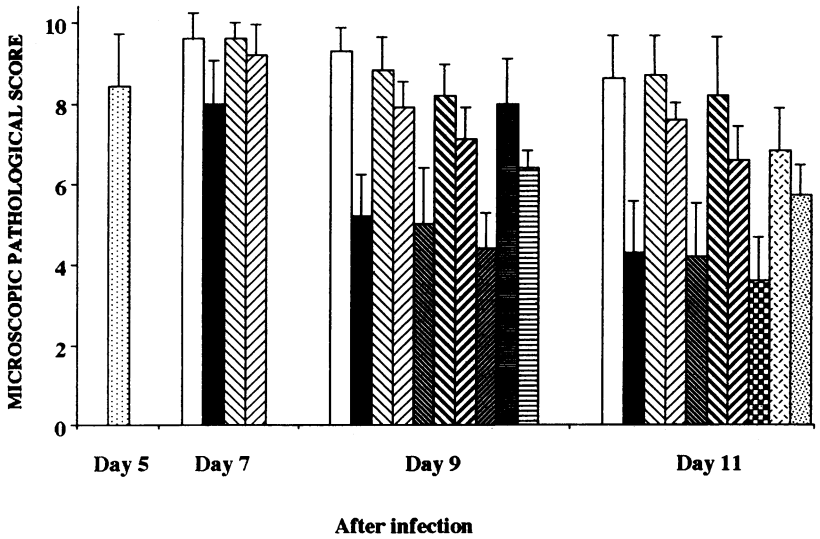

FIG. 4. Course of microscopic severity of experimentally induced *M. pneumoniae* pneumonia in hamsters. Results are means for five hamsters per group with standard deviations. Column numbers, counting from left to right: 1, early control; 2, untreated control; 3, azithromycin at 10 mg/kg once daily for 1 day; 4, erythromycin at 10 mg/kg once daily for 1 day; 5, clarithromycin at 10 mg/kg once daily for 1 day; 10, azithromycin at 10 mg/kg once daily for 3 days; 11, erythromycin at 10 mg/kg once daily for 3 days; 12, clarithromycin at 10 mg/kg once daily for 3 days; 13, azithromycin at 15 mg/kg twice daily for 2.5 days; 14, erythromycin at 15 mg/kg twice daily for 2.5 days; 15, clarithromycin at 15 mg/kg twice daily for 2.5 days; 23, azithromycin at 15 mg/kg twice daily for 5 days; 24, erythromycin at 15 mg/kg twice daily for 5 days; 25, clarithromycin at 15 mg/kg twice daily for 5 days.

days ($P < 0.0005$), and at 15 mg/kg twice daily for 5 days ($P < 0.0005$) were effective.

On day 11 after infection, all regimens of azithromycin were more effective than the corresponding regimens of erythromycin and clarithromycin ($P < 0.01$).

Levels of azithromycin, erythromycin, and clarithromycin in hamster lungs. Levels of azithromycin, erythromycin, and clarithromycin in lungs are shown in Table 5. The peak concentrations of azithromycin in lungs after administration of a single oral dose of 50 mg/kg were the same as that of clarithromycin and far higher than that of erythromycin. The half-life of azithromycin in lungs was about 15 times longer than those of erythromycin and clarithromycin. The high concentration and long half-life of azithromycin in hamster lungs suggest that azithromycin might be expected to have a good therapeutic effect against *M. pneumoniae* infection.

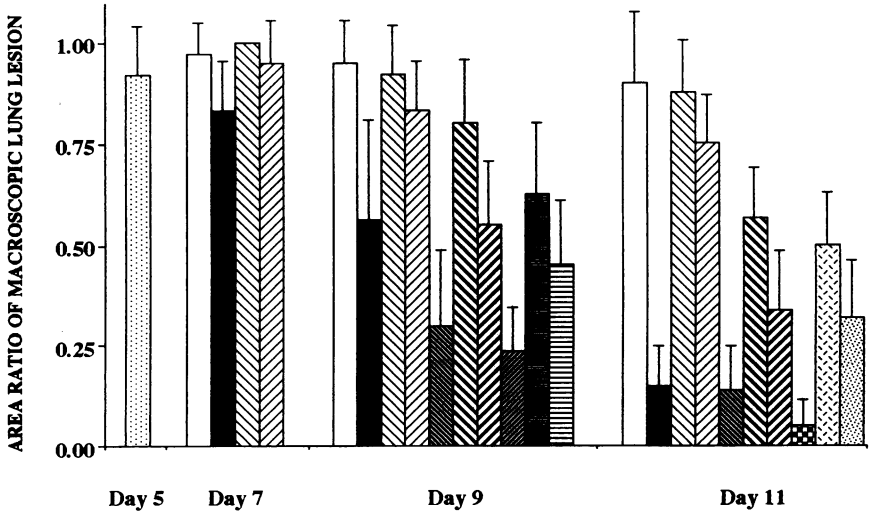

FIG. 5. Course of macroscopic severity of experimentally induced *M. pneumoniae* pneumonia in hamsters. Results are means for 10 hamsters per group with standard deviations. Column numbers, counting from left to right: 1, early control; 2, untreated control; 3, azithromycin at 10 mg/kg once daily for 1 day; 4, erythromycin at 10 mg/kg once daily for 1 day; 5, clarithromycin at 10 mg/kg once daily for 1 day; 10, azithromycin at 10 mg/kg once daily for 3 days; 11, erythromycin at 10 mg/kg once daily for 3 days; 12, clarithromycin at 10 mg/kg once daily for 3 days; 13, azithromycin at 15 mg/kg twice daily for 2.5 days; 14, erythromycin at 15 mg/kg twice daily for 2.5 days; 15, clarithromycin at 15 mg/kg twice daily for 2.5 days; 23, azithromycin at 15 mg/kg twice daily for 5 days; 24, erythromycin at 15 mg/kg twice daily for 5 days; 25, clarithromycin at 15 mg/kg twice daily for 5 days.

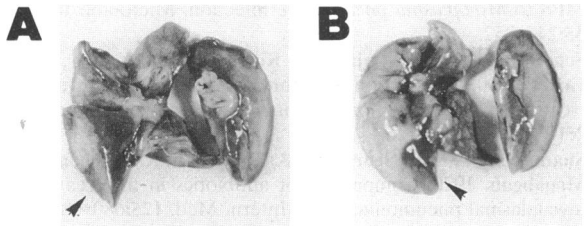

FIG. 6. Macroscopic findings in hamster lungs on day 11 after infection with *M. pneumoniae*. (A) Untreated control. Clear consolidation occupying about 75% or more of the right lower lobe is observed (arrowhead). (B) Treatment with azithromycin at 10 mg/kg once daily for 3 days. Consolidation in the right lower lobe is definitely reduced (arrowhead).

DISCUSSION

Recently developed macrolide antibacterial agents, such as azithromycin, clarithromycin, and roxithromycin, have broad and potent antibacterial activities and have been applied to various kinds of infections in humans (8). Some reports suggest that the new macrolides have good activities against *M. pneumoniae* (5, 13, 14, 16). The current therapy against *M. pneumoniae* pneumonia is mainly erythromycin, but some investigators have reported that azithromycin was as effective as erythromycin (18). MICs of azithromycin and other macrolides have been reported (5, 13, 14, 16), but not the definite range of azithromycin MICs. In our study, the MIC of azithromycin for 90% of strains was 1/32 (or less) of those of erythromycin, clarithromycin, and roxithromycin. This result suggests that azithromycin may be more effective than other macrolides.

Fourteen- and 16-membered-ring macrolides, such as erythromycin, clarithromycin, roxithromycin, josamycin, and leukomycin, have lower activities than azithromycin against gram-negative bacteria and *M. pneumoniae* (8, 9, 12). Although *M. pneumoniae* is phylogenetically related to gram-positive bacteria and not to gram-negative bacteria, it seems that the susceptibility of *M. pneumoniae* to macrolides roughly parallels that seen with gram-negative bacteria. In our in vivo study, two different parameters, i.e., viable mycoplasmal cell counts and histopathological severity, were used for estimating efficacies of therapy with azithromycin, clarithromycin, and erythromycin. Histopathological features in humans and hamsters are similar (11), so our in vivo data should be more interesting than those from studies that only count the numbers of *M. pneumoniae* in order to estimate drug efficacy.

There was no statistically significant histopathological difference between the early control (day 5 after infection) and the untreated control on days 7, 9, and 11, which showed that there was no spontaneous histological reduction in the hamsters' mycoplasmal pneumonia. On the other hand, the number of viable *M. pneumoniae* cells in hamster lungs on day 7 after infection increased significantly, that on day 9 decreased compared with that on day 7, and that on day 11 also decreased significantly compared with that on day 9. These results show that the natural histopathological course in the hamsters' mycoplasmal pneumonia was not parallel with the natural course of viable *M. pneumoniae* numbers in the hamsters' lungs; that is, the histopathological change in the lungs remains after reduction of the number of viable *M. pneumoniae* cells. On the basis of our therapeutic study, in which erythromycin at 10 mg/kg once daily for 3 days and clarithromycin at 10 mg/kg once daily for 1 day (Table 4) reduced histopathological severities significantly and did not reduce the numbers of mycoplasma cells in lungs on day 9 after infection, macrolide therapy seems to be more effective for reductions in histopathologies than for the reduction of numbers of viable *M. pneumoniae* cells. This may reflect the mechanism of action of macrolide antibiotics, which is a bacteriostatic activity; that is, macrolides in some concentrations only inhibit the virulence process of *M. pneumoniae*, which is complex, without killing *M. pneumoniae* (21).

Azithromycin was significantly more effective than erythromycin or clarithromycin in the same regimens. Especially at 10 mg/kg once daily for 1 day, only azithromycin was significantly effective in reducing viable mycoplasma cell counts and histopathologies (Table 4). Good pharmacokinetic activity in hamsters and excellent antimycoplasmal potency are why azithromycin showed greater efficacy than other drugs in the in vivo study using a hamster pulmonary infection model. We cannot prove the efficacy of azithromycin in treating *M. pneumoniae* pulmonary infection in humans, but it is generally recognized that *M. pneumoniae* pneumonia in hamsters is similar to that in humans, so we can guess that a smaller dose of azithromycin than of erythromycin or clarithromycin may be effective in *M. pneumoniae* pneumonia in humans. Our data suggest that clinical studies of macrolides in human patients are warranted.

TABLE 5. Pharmacokinetics of azithromycin, erythromycin, and clarithromycin in lungs after a single oral dose of 50 mg/kg[a]

Drug	C_{max} (μg/g) (mean ± SD)	T_{max} (h)	$t_{1/2}$ (h)
Azithromycin	28.5 ± 15.5	1.0	22.5
Erythromycin	0.483 ± 0.113	1.0	1.6
Clarithromycin	34.0 ± 9.1	2.0	1.4

[a] Abbreviations: C_{max}, maximum concentration; T_{max}, time to maximum concentration; $t_{1/2}$, half-life.

REFERENCES

1. **Azoulay-Dupuis, E., E. Vallee, J. P. Bedos, M. Muffat-Joly, and J.-J. Pocidalo.** 1991. Prophylactic and therapeutic activities of azithromycin in a mouse model of pneumococcal pneumonia. Antimicrob. Agents Chemother. **35**:1024–1028.
2. **Barile, M. F., D. K. F. Chandler, H. Yoshida, M. W. Grabowski, R. Hayasawa, and S. Razin.** 1988. Parameters of *Mycoplasma pneumoniae* infection in Syrian hamsters. Infect. Immun. **56**:2443–2449.
3. **Chanock, R. M., L. Hayflick, and M. F. Barile.** 1962. Growth on artificial medium of an agent associated with atypical pneumonia and its identification as PPLO. Proc. Natl. Acad. Sci. USA **48**:41–49.
4. **Clyde, W. A., Jr.** 1964. Mycoplasma species identification based upon growth inhibition by specific antisera. J. Immunol. **92**:958–965.
5. **Felmingham, D., M. J. Robbins, M. Sanghrajka, A. Leakey, and G. L. Ridgway.** 1991. The in vitro activity of some 14-, 15- and 16-membered macrolides against *Staphylococcus spp.*, *Legionella spp.*, *Mycoplasma spp.* and *Ureaplasma urealyticum*. Drugs Exp. Clin. Res. **17**:91–99.
6. **Foy, H. M., G. E. Kenny, M. K. Cooney, and I. D. Allen.** 1979. Long-term epidemiology of infections with *Mycoplasma pneumoniae*. J. Infect. Dis. **139**:681–687.
7. **Girard, A. E., D. Girard, A. R. English, T. D. Gootz, C. R. Cimochowski, J. A. Faiella, S. L. Haskell, and J. A. Retsema.** 1987. Pharmacokinetic and in vivo studies with azithromycin (CP-62,933), a new macrolide with an extended half-life and excellent tissue distribution. Antimicrob. Agents Chemother. **31**:1948–1954.
8. **Hardy, D. J., D. M. Hensey, J. M. Beyer, C. Vojtko, E. J. McDonald, and P. B. Fernandes.** 1988. Comparative in vitro activities of new 14-, 15-, and 16-membered macrolides. Antimicrob. Agents Chemother. **32**:1710–1719.

9. **Kenny, G. E., and F. D. Cartwright.** 1991. Susceptibility of *Mycoplasma pneumoniae* to several new quinolones, tetracycline, and erythromycin. Antimicrob. Agents Chemother. **35**:587–589.
10. **Kirst, H. A., and G. D. Sides.** 1989. New directions for macrolide antibiotics: pharmacokinetics and clinical efficacy. Antimicrob. Agents Chemother. **33**:1419–1422.
11. **Krause, D. C., and D. T. Robinson.** 1992. Mycoplasmas which infect humans, p. 417–444. *In* J. Maniloff, R. N. McElhaney, L. R. Finch, and J. B. Baseman (ed.), Mycoplasmas: molecular biology and pathogenesis. American Society for Microbiology, Washington, D.C.
12. **Osada, Y., and H. Ogawa.** 1983. Antimycoplasmal activity of ofloxacin (DL-8280). Antimicrob. Agents Chemother. **23**:509–511.
13. **Renaudin, H., and C. Bebear.** 1990. Comparative in vitro activity of azithromycin, clarithromycin, erythromycin and lomefloxacin against *Mycoplasma pneumoniae*, *Mycoplasma hominis* and *Ureaplasma urealyticum*. Eur. J. Clin. Microbiol. Infect. Dis. **9**:839–841.
14. **Renaudin, H., J. G. Tully, and C. Bebear.** 1992. In vitro susceptibilities of *Mycoplasma genitalium* to antibiotics. Antimicrob. Agents Chemother. **36**:870–872.
15. **Retsema, J., A. Girard, W. Schelkly, M. Manousos, M. Anderson, G. Bright, R. Borovoy, L. Brennan, and R. Mason.** 1987. Spectrum and mode of action of azithromycin (CP-62,993), a new 15-membered-ring macrolide with improved potency against gram-negative organisms. Antimicrob. Agents Chemother. **31**:1932–1947.
16. **Rylander, M., and H. O. Hallander.** 1988. In vitro comparison of the activity of doxycycline, tetracycline, erythromycin and a new macrolide, CP 62993, against *Mycoplasma pneumoniae*, *Mycoplasma hominis* and *Ureaplasma urealyticum*. Scand. J. Infect. Dis. Suppl. **53**:12–17.
17. **Sasaki, Y., A. Ogura, K. Nakayama, Y. Noguchi, K. Matuno, and M. Saito.** 1991. Susceptibility of newly established mouse strain MPS to *Mycoplasma pneumoniae* infection. Microbiol. Immunol. **35**:247–252.
18. **Schönwald, S., M. Gunjača, L. Kolacny-Babić, V. Car, and M. Gošev.** 1990. Comparison of azithromycin and erythromycin in the treatment of atypical pneumonias. J. Antimicrob. Chemother. **25**(Suppl. A):123–126.
19. **Shames, J. M., R. B. George, W. B. Holliday, J. R. Rasch, and W. J. Mogabgab.** 1970. Comparison of antibiotics in the treatment of mycoplasmal pneumonia. Arch. Intern. Med. **125**:680–684.
20. **Slotkin, R. I., W. A. Clyde, Jr., and F. W. Denny.** 1967. The effect of antibiotics on *Mycoplasma pneumoniae* in vitro and in vivo. Am. J. Epidemiol. **86**:225–237.
21. **Tryon, V. V., and J. B. Baseman.** 1992. Pathogenetic determinants and mechanisms, p. 457–471. *In* J. Maniloff, R. N. McElhaney, L. R. Finch, and J. B. Baseman (ed.), Mycoplasmas: molecular biology and pathogenesis. American Society for Microbiology, Washington, D.C.
22. **Waites, K. B., G. H. Cassel, K. C. Canupp, and P. B. Fernandes.** 1988. In vitro susceptibilities of mycoplasmas and ureaplasmas to new macrolides and aryl-fluoroquinolones. Antimicrob. Agents Chemother. **32**:1500–1502.
23. **Waites, K. B., L. B. Duffy, T. Schmid, D. Crabb, M. S. Pate, and G. H. Cassel.** 1991. In vitro susceptibilities of *Mycoplasma pneumoniae*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* to sparfloxacin and PD 127391. Antimicrob. Agents Chemother. **35**:1181–1185.
24. **Waitz, J. A., G. V. Doern, S. M. Finegold, T. L. Gavan, J. L. Hakett, R. N. Jones, J. H. Jorgensen, J. R. King, G. H. Miller, L. B. Reller, C. Thornsberry, L. D. Thrupp, and R. J. Zabransky.** 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 2nd ed. NCCLS publication no. M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.