Efficacy of Cethromycin, a New Ketolide, against *Streptococcus pneumoniae* Susceptible or Resistant to Erythromycin in a Murine Pneumonia Model

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Cethromycin is a ketolide with in vitro activity against macrolide-sensitive and -resistant strains of *Streptococcus pneumoniae*. We compared its in vivo efficacy to erythromycin in a mouse model of acute pneumonia induced by two virulent clinical strains: a serotype 3 susceptible strain (P-4241) (MICs: erythromycin, 0.03 µg/ml; cethromycin, 0.015 µg/ml) and a serotype 1 strain resistant to erythromycin (P-6254; phenotypically MLSB constitutive) (MICs: erythromycin, 1,024 µg/ml; cethromycin, 0.03 µg/ml). Immunocompetent mice were infected with 10⁵ CFU of each strain. Six treatments given either subcutaneously (s.c.) or per os (p.o.) at 12-h intervals were initiated at 6 or 12 h after infection. Against P-4241, cethromycin given s.c. at 25 or 12.5 mg/kg protected 100% of the animals, with lungs and blood completely cleared of bacteria. Given p.o., cethromycin maintained its efficacy with 100 and 86% survival at 25 and 12.5 mg/kg, respectively. Erythromycin, given s.c. at 50 or 37.5 mg/kg, provided 50 and 38% survival rates, respectively. Against P-6254, cethromycin was effective at 25 mg/kg (100% survival) regardless of the administration route, whereas only 25 and 8% of animals survived after a 75-mg/kg erythromycin treatment given s.c. and p.o., respectively. The serum protein binding levels of cethromycin were 94.8 and 88.5% after doses of 12.5 and 25 mg/kg, respectively. The higher in vivo activity of cethromycin compared to erythromycin could be explained by favorable pharmacokinetic/pharmacodynamic indexes against P-6254 but not against P-4241.

Community-acquired pneumonia is the most common cause of death due to infection, with *Streptococcus pneumoniae* remaining the leading pathogen (17, 21, 26). The number of pneumococcal strains resistant to penicillin, macrolides, and other antimicrobials has increased at an alarming rate during the past two decades. The incidence of erythromycin resistance is reported to be as high as 20% in Quebec, Canada (31), and 55% in France (9). *S. pneumoniae* strains resistant to erythromycin are also considered to be resistant to all other macrolides. There is considerable interest in the use of alternative antimicrobials, such as ketolides active against macrolide-resistant strains.

The ketolides, semisynthetic 14-membered ring macrolides, represent a new subclass of agents in the macrolide-lincosamide-streptogramin group. The substitution of the L-cladinose sugar with a 3-keto group on the erythronolide A ring is the major differing structural component of the ketolides (7). Macrolide resistance in *S. pneumoniae* is predominantly mediated by two mechanisms. The first mechanism of resistance, referred to as the MLSB phenotype (for macrolides, lincosamide, and streptogramin B), is due to the presence of the *ermB* gene, coding for a ribosomal methylase resulting in a posttranslational modification of 23S rRNA. These strains are resistant to 14-membered macrolides such as erythromycin, clarithromycin, and roxithromycin, 15-membered azalides such as azithromycin, and 16-membered macrolides such as josamycin and spiramycin, as well as the lincosamide clindamycin. Resistance to macrolides may be constitutively expressed or

induced by sub-MICs of the macrolide (7, 30). The second mechanism of resistance, referred to as the M phenotype, is found in strains containing the *mef* genes coding for an efflux pump; these strains are resistant to 14-membered macrolides and azalides but are susceptible to 16-membered macrolides, as well as clindamycin. In addition, mutations in 23S rRNA domains II and V and ribosomal proteins L4 and L22 associated with macrolide resistance have also been determined (29). Due to enhanced accumulation into cells and tighter ribosomal binding, ketolides maintain activity against *mef*- and/or *ermB*-containing *S. pneumoniae* (7). Moreover, ketolides showed a second ribosomal binding site at domain V, as well as at domain II of the 23S RNA subunit (13). Cethromycin (ABT-773), one of the newest agents, has a high in vitro activity against a variety of resistant phenotypes, including activity against penicillin- and macrolide-resistant gram-positive bacteria (15, 18, 24).

The purpose of the present study was to compare the efficacy of cethromycin and erythromycin against a macrolide-resistant and a macrolide-susceptible strain in a mouse model of acute *S. pneumoniae* pneumonia.

MATERIALS AND METHODS

Bacterial strains. Strain P-4241 is a blood isolate (serotype 3), capsulated and virulent in a mouse model of acute pneumonia (100% lethal dose = 3.34 log₁₀ CFU/mouse). This strain is susceptible to both penicillin (MIC = 0.015 µg/ml) and erythromycin (MIC = 0.015 µg/ml). Strain P-6254 is also a virulent (100% lethal dose = 3.11 log₁₀ CFU/mouse) blood isolate (serotype 1). This strain is susceptible to penicillin (MIC = 0.03 µg/ml) and constitutively resistant to erythromycin (MIC = 1,024 µg/ml) and other macrolides, lincosamide, and streptogramin B (i.e., the MLSB phenotype) (2).

MIC determinations. MICs were determined by the broth microdilution method in Columbia medium supplemented with 5% sheep blood (23).

Drugs. The antibiotics used in the present study included cethromycin (Abbott Laboratories, Chicago, IL) and erythromycin (Pfizer Laboratories, Groton, CT). Cethromycin was solution formulated the same for both subcutaneous (s.c.) and oral administration in 2% ethanol with 1 mol eq of HCl (vol/vol) in 5% sterile

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TABLE 1. PK indexes for serum and lung tissues of control and P-4241-infected mice after administration of a single s.c. or oral dose of cethromycin^a

Mouse group (dose [mg/kg]) and compartment	Mean $C_{\max}$ ($\mu\text{g/ml}$ or $\mu\text{g/g}$) $\pm$ SD	Mean $T_{\max}$ (h) $\pm$ SD	$C_{24\text{ h}}$ ($\mu\text{g/ml}$ or $\mu\text{g/g}$)	Mean AUC ₀₋₂₄ ($\mu\text{g} \cdot \text{h/ml}$ or $\mu\text{g} \cdot \text{h/g}$) $\pm$ SD	Mean $t_{1/2}$ (h) $\pm$ SD
Uninfected control (12.5, s.c.)					
Serum	1.2 $\pm$ 0.3	1.4 $\pm$ 1.0	0.01	2.7 $\pm$ 0.6	1.7 $\pm$ 0.9
Lung	12.1 $\pm$ 4.8	2.0 $\pm$ 0.9	0.2	30.1 $\pm$ 11.6	3.0 $\pm$ 0.8
Infected (12.5, s.c.)					
Serum	1.3 $\pm$ 0.4	0.6 $\pm$ 0.2	0.01	3.1 $\pm$ 0.3	1.5 $\pm$ 0.3
Lung	10.8 $\pm$ 3.0	1.6 $\pm$ 0.7	0.3	36.3 $\pm$ 8.7	2.7 $\pm$ 0.8
Infected (25, oral)					
Serum	1.8 $\pm$ 0.3	1.8 $\pm$ 0.7	0.001	10.3 $\pm$ 2.9	1.4 $\pm$ 0.6
Lung	16.4 $\pm$ 4.6	2.8 $\pm$ 1.0	0.3	86.1 $\pm$ 24.4	4.4 $\pm$ 0.8

^a Concentration values are total drug concentrations in sera and lungs. Values for cethromycin are means and standard deviations calculated from data for six serum and lung tissue samples taken at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h postdosing.

^b That is, the total drug concentration in serum and lungs at 24 h posttreatment.

dextrose water (pH 5) (20). Erythromycin was diluted in sterile water to the desired concentrations.

Infection of mice with *S. pneumoniae*. Animal studies were performed in accordance with prevailing regulations regarding the care and use of laboratory animals of the European Commission (11). Swiss mice (20 to 22 g; Ifa Credo, L'Arbresle, France) were infected by the intratracheal route with 40 μl of bacterial suspension at a dose of $\sim 10^5$ CFU (1).

Antibiotic treatment. Therapy was initiated 12 h after challenge with P-4241 and 6 h after challenge with P-6254. Early treatment initiation was required for P-6254 because mice infected with this strain developed pneumonia within 48 h after infection. Cethromycin was given as six injections, either given s.c. or by gavage at 12-h intervals, at doses of 6.25, 12.5, 25, and 50 mg/kg to mice challenged with either strain P-4241 or strain P-6254 (16 treatment groups). Erythromycin was given as six injections at 12-h intervals given s.c. at 50 mg/kg and either s.c. or by gavage at a dose of 37.5 mg/kg to mice challenged with strain P-4241 and at doses of 75 and 150 mg/kg to mice challenged with strain P-6254 (seven treatment groups). Each treatment group comprised between 12 to 16 animals. Survival experiments were also performed after two, four, or six s.c. injections of 6.25 or 12.5 mg of cethromycin/kg and 50 mg of erythromycin/kg. The observation period was 10 days. Death rates were recorded daily, and the cumulative survival rates were compared.

Bactericidal activity in vivo. The protocol used to study the bactericidal activity was the same as that used for the mouse survival studies. Animals were infected with P-6254, and the first treatment was initiated 6 h after bacterial challenge. The total CFU counts recovered from whole-lung homogenates were determined 12 h after the first, second, fourth, and sixth administrations at doses of 6.25, 12.5, and 25 mg of cethromycin/kg. Three mice were used for each dose and time point. Mice were killed by intraperitoneal injection of sodium pentobarbital and were exsanguinated by cardiac puncture; blood was used for the cultures. The lungs were removed and homogenized in 1 ml of normal saline. A total of 100 μl of whole lung homogenate or serial 10-fold dilutions of homogenates were plated on Columbia agar. The limit of detection was equal to 1 log₁₀ CFU/ml. Blood was cultured in brain heart infusion broth. After overnight culture, colonies were counted on agar plates seeded with lung samples, blood cultures were examined for turbidity, and a qualitative result was obtained. The results are expressed as the mean $\pm$ the standard deviation log₁₀ CFU per lung and as the number of positive or negative blood cultures for groups of three mice each.

Analysis of strains recovered from mice. Bacteria were recovered from homogenized lungs of treated and untreated control animals. Lung homogenates (100 μl) were spread on Columbia agar plates supplemented with 5% sheep blood. Approximately 10 individual colonies were isolated at random after overnight culture at 37°C, and their resistance profiles were determined.

Determination of cethromycin and erythromycin concentrations in serum and lung and pharmacokinetic (PK) analysis. (i) **Samples.** PK studies were performed in Swiss mice treated with cethromycin either given s.c. at a single dose of 12.5 mg/kg in healthy controls and P-4241-infected animals or orally at 25 mg/kg dose in P-4241-infected animals. PK studies were also performed in mice treated with erythromycin after a single dose of 50 mg/kg given s.c. in healthy controls and in P-4241-infected animals. Infected mice were treated at 12 h postinfection. Serum and lung samples were collected from groups of six mice each at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after drug administration. All samples

were frozen at -70°C and protected from light to avoid antibiotic degradation during analysis. Lung tissue samples were crushed in liquid nitrogen by magnetized crushing (Spex; Fisher Bioblock, Illkirch, France).

(ii) **Cethromycin assays.** Preparation of the calibration standard started with a standard stock solution. Approximately 25 mg of cethromycin was placed into a labeled, polypropylene volumetric flask. Calibration standards from 0.02 to 4.0 $\mu\text{g/ml}$ (serum) and from 0.5 to 50.0 $\mu\text{g/g}$ (lung) were prepared with diluted stock solutions by spiking mouse serum and lung homogenates, respectively. A 0.5 M Na₂CO₃ solution (500 μl) and internal standard methanolic solution (A-207257; 1 g/liter, 50 μl) were added to serum (200 μl) and lung homogenate (20 mg of crushed powder, accurately weighed) samples, respectively. After being mixed with 6 ml of ethyl acetate-hexane (1:1 by volume) and centrifugation (1,000 $\times$ g, 10 min), the organic layer was evaporated to dryness with nitrogen at room temperature and protected from light. Dry samples were reconstituted with acetonitrile–0.05 M phosphate buffer (pH 6.0) (1:2 by volume). Extracted samples of 50 μl were automatically injected onto a Symmetry column (C₁₈, 4.6 by 250 mm, 5 μm ; Waters, Milford, MA) coupled with a spectrofluorometric detection (324 and 364 nm for excitation and emission, respectively). The mobile phase was a mixture of acetonitrile, methanol, and buffer mobile phase (45:5:60, by volume) at a flow rate of 1.0 ml/min. The buffer mobile phase (pH 6.0) contained 0.01 M tetramethylammonium hydroxide–0.05 M KH₂PO₄. The method was validated over the respective calibration ranges; the between-day and within-day coefficients of variation were lower than 10% in both serum and lung samples, and the limits of quantification were 0.02 $\mu\text{g/ml}$ and 0.25 $\mu\text{g/g}$ in serum and lung samples, respectively. Cethromycin-unbound drug was separated from serum samples by ultrafiltration using a Centrifree centrifugal filter device (Amicon Bioseparations; Millipore Corp., Bedford, MA). Serum samples were collected at 1.5 h posttreatment from two groups of six healthy control Swiss mice treated with cethromycin given at a single dose of either 12.5 mg/kg s.c. or 25 mg/kg orally. Then, a 250- μl aliquot of serum samples was transferred to the Centrifree device. The ultrafiltration by centrifugation was conducted at room temperature for 20 min at 1,500 $\times$ g. A 50- μl aliquot of ultrafiltrate was injected directly in the chromatographic system. Calibration standards from 0.02 to 4.0 $\mu\text{g/ml}$ were prepared with diluted stock solutions in deionized water.

(iii) **Erythromycin assays.** Erythromycin concentrations were determined by the agar well diffusion method of bioassay using *Micrococcus luteus* (ATCC 9341) as the bioassay organism and Antibiotic Medium 11 (Difco Laboratories, Detroit, MI) as the growth medium. The sensitivity of the assay was 0.1 $\mu\text{g/ml}$, and the relative error of the assay was $<10\%$.

(iv) **PK analysis.** PK analysis was based on a noncompartmental model (WinNonlin, version 1.1; Pharsight, Mountain View, CA). Maximum concentrations in serum ($C_{\max}$) and time above MIC were measured experimentally, whereas the area under the concentration-time curve from 0 to 24 h (AUC₀₋₂₄) and terminal half-life ($t_{1/2}$) were calculated by using WinNonlin software.

RESULTS

In vitro activity of cethromycin. The MIC/MBC values for erythromycin were 0.03/0.125 $\mu\text{g/ml}$ against P-4241 and 1,024/ $>1,024$ $\mu\text{g/ml}$ against P-6254. The MIC/MBC values of cethro-

TABLE 2. PK indexes for serum and lung tissues of control and P-4241-infected mice after the administration of a single 50-mg/kg s.c. dose of erythromycin^a

Mouse group and compartment	C _{max} (μg/ml or μg/g)	T _{max} (h)	AUC ₀₋₂₄ (μg · h/ml or μg · h/g)	t _{1/2} (h)
Uninfected control (s.c.)				
Serum	5.9	0.5	12	0.8
Lung	16.1	1	36	0.8
Infected (s.c.)				
Serum	7.1	0.5	12	0.8
Lung	17.2	0.5	34	0.9

^a Drug concentration values are total drug concentrations in serum and lungs. Values were calculated from data for six serum samples taken at 0.5, 1, 2, 4, 8, 12, and 24 h postdosing.

mycin were 0.015/0.015 and 0.03/0.5 μg/ml against the susceptible and resistant strains, respectively.

In vivo studies: PK data. Relevant serum and lung PK data for cethromycin and erythromycin are given in Tables 1 and 2, respectively. Values are given for total drug concentrations in serum and lung samples after a single dose for each antibiotic. When the antibiotics were administered either to healthy controls or to P-4241-infected animals, whatever the route of administration (s.c. or oral), cethromycin exhibited a prolonged *t*_{1/2} in sera (1.4 to 1.7 h) and lungs (2.7 to 4.4 h) compared to erythromycin (0.8 h and 0.8 to 0.9 h in sera and lungs, respectively). The AUCs in lungs of control and P-4241-infected animals were similar between cethromycin at 12.5 mg/kg given s.c. (30.1 and 36.3 μg · h/ml, respectively) and erythromycin at 50 mg/kg given s.c. (36 and 34 μg · h/ml, respectively). The ratio of tissue AUC to serum AUC was greater with cethromycin (mean value of 11 versus 3 for erythromycin), suggesting an accumulation of cethromycin in lungs. Pharmacodynamic (PD) indexes are shown in Table 3. Values are given for total and free drug concentrations. Against strain P-4241, for a 50-mg/kg dose of erythromycin given s.c. the serum AUC/MIC ratio, equal to 400, was intermediate between a 12.5 mg/kg (AUC/MIC = 207) and a 25-mg/kg (AUC/MIC = 687) dose of

cethromycin given s.c., whereas in lung tissue it was lower (AUC/MIC = 1,133) compared to a 12.5-mg/kg dose of cethromycin (AUC/MIC = 2,420). The serum protein binding values for cethromycin were 94.8 and 88.5% for 12.5- and 25-mg/kg doses given s.c. and orally, respectively. Therefore, by considering a free drug concentration of erythromycin of 80%, the serum AUC_{free}/MIC value was larger with erythromycin at 50 mg/kg (AUC_{free}/MIC = 320) than with cethromycin (AUC_{free}/MIC = 10.8 and 79 at 12.5 and 25 mg/kg, respectively). Similarly, in lungs, the AUC_{free}/MIC ratios were 906 after a 50-mg/kg dose of erythromycin and 660 after a 25-mg/kg dose of cethromycin. Times during which free serum concentrations were above MIC (serum-free T>MIC) against the susceptible strain were intermediate after a 50-mg/kg dose of erythromycin compared to those obtained after a 12.5- or a 25-mg/kg dose of cethromycin, respectively.

For the resistant strain P-6254, the PK and PD indices of erythromycin were negligible compared to those of cethromycin.

(ii) Survival. All untreated control mice died within 5 days after challenge with P-4241 and within only 2 days after challenge with P-6254. When mice were infected with the macrolide-susceptible strain P-4241 (Fig. 1A), six s.c. injections of cethromycin at 12.5, 25, and 50 mg/kg yielded 100% survival. Even at the lowest dose of 6.25 mg/kg, an 86% survival rate was observed. Cethromycin administered by gavage was also effective, with survival rates equal to 100, 86, and 80%, at 25-, 12.5-, and 6.25-mg/kg doses, respectively. In comparison, much lower survival rates were observed with erythromycin (Fig. 1C), even when it was given at higher dosages than cethromycin, since six injections of 50 and 37.5 mg/kg administered s.c. provided survival rates of 50 and 38%, respectively, and of 0% when administered by gavage at 37.5 mg/kg. Cethromycin exhibited higher survival rates even after two s.c. injections, with 54 and 69% survivals at 6.25 and 12.5 mg/kg, respectively, compared to macrolide, with 27% survival at 50 mg/kg (Table 4).

When mice were infected with the macrolide-resistant strain P-6254 (Fig. 1B), the survival rates after cethromycin treatment remained relatively high (100, 86, and 71% at s.c. doses of 25, 12.5, and 6.25 mg/kg, respectively) versus 100, 38, and 14% after

TABLE 3. Relationship between PK and PD indexes in serum and survival of P-4241- and P-6254-infected mice after single s.c. or orally administered doses of cethromycin or erythromycin

Mouse group and dose (mg/kg)	Serum		Lung		Serum-free T>MIC		% Survival
	AUC/MIC	AUC _{free} /MIC	AUC/MIC	AUC _{free} /MIC	Time (h)	%	
Infected with P-4241, treated with cethromycin							
12.5, s.c.	207	10.8	2,420	126	3.7	15	100
25, oral	687	79	5,740	660	16.2	67	100
Infected with P-6254, treated with cethromycin							
12.5, s.c.	103	5.4	1,210	63	3.4	14	86
25, oral	343	39	2,870	330	11	46	100
Infected with P-4241, treated with erythromycin							
50, s.c.	400	320	1,133	906	6.5	27	50
Infected with P-6254, treated with erythromycin							
50, s.c.	<<0.1	<<0.08	0	0	0	0	0

^a Protein binding was found to be equal to 94.8% at 12.5 mg/kg (free fraction [ff], 5.2%) and 88.5% at 25 mg/kg (ff, 11.5%) for cethromycin and was considered to be 20% for erythromycin (ff, 80%) (10, 28).

FIG. 1. (A) Survival of mice challenged with the penicillin- and macrolide-susceptible strain P-4241 and treated with 6.25, 12.5, or 25 mg of cethromycin/kg. The antibiotic was given either s.c. or by gavage (G). The AUC_{free}/MIC ratios after a single dose of 12.5 (s.c.) and 25 (G) mg/kg are 10.8 and 79, respectively. (B) Survival of mice challenged with the penicillin-susceptible and macrolide-resistant strain P-6254 and treated with 6.25, 12.5, or 25 mg of cethromycin/kg. The antibiotic was given either s.c. or by gavage (G). The AUC_{free}/MIC ratios after a single dose of 12.5 (s.c.) and 25 mg/kg (gavage) are 5.4 and 39, respectively. (C) Survival of mice challenged with a penicillin- and macrolide-susceptible strain P-4241 and treated with 37.5 or 50 mg of erythromycin/kg. The antibiotic was given either s.c. or by gavage (G). The AUC_{free}/MIC ratio after a single dose of 50 mg/kg (s.c.) is 320. (D) Survival of mice challenged with the penicillin-susceptible and macrolide-resistant strain P-6254 and treated with 75 or 150 mg of erythromycin/kg. The antibiotic was given either s.c. or by gavage (G). The AUC_{free}/MIC ratio is 0.

gavage, respectively, with the same dosages. Survival rates dropped dramatically with erythromycin (Fig. 1D) and reached 25% (s.c.) and 8% (gavage) after a 75-mg/kg treatment. None of the animals survived at a 150-mg/kg dose given either s.c. or by gavage, probably indicating a toxic effect of erythromycin.

Bacterial clearance. Bacterial growth in the lungs of mice infected with strain P-4241 was observed from 21 h postinfection ($4.3 \log_{10}$ CFU/ml) until death at 88 h postinfection ($8 \log_{10}$ CFU/ml). Blood cultures were always positive in untreated controls. The lungs and blood of mice infected with strain P-4241 were completely cleared after one injection of cethromycin at 25 or 12.5 mg/kg and two (lungs) and four (blood) injections at 6.25 mg/kg. No bacterial regrowth was observed 12 h in both lungs and blood after six doses. In animals infected with strain P-6254, the lungs and blood were completely cleared after, respectively, four cethromycin injections at 25 mg/kg or six cethromycin injections at 12.5 and 6.25

mg/kg (Table 5). Bacterial clearances were in agreement with these survival rates.

In vivo emergence of resistance. Colonies isolated at the end of treatment from untreated controls and from erythromycin- and cethromycin-treated animals infected by P-4241 or P-6254 maintained the same antibiotic profile as the inoculated strain.

DISCUSSION

Our in vivo data showed that cethromycin was far more potent than erythromycin against the macrolide-resistant strain, a constitutive MLSB phenotype, with 100% survival at 12.5 mg/kg s.c. and 25 mg/kg orally versus 25% survival at 75 mg/kg s.c., for erythromycin. This result was mainly due to the difference in in vitro activities with MICs of 0.03 and $>1,024 \mu\text{g/ml}$ for cethromycin and erythromycin, respectively, which resulted in terms of PK and PD indexes in serum or lung AUC_{free}/MIC

TABLE 4. Survival rates at 2, 4, and 10 days postinfection after 1, 2, or 3 days of s.c. treatment of mice infected with a penicillin- and macrolide-susceptible strain (P-4241)

Drug (dosage [mg/kg])	% Survival in experimental pneumococcal pneumonia after antibiotic treatment for:								
	One day (two doses)			Two days (four doses)			Three days (six doses)		
	Day 2	Day 4	Day 10	Day 2	Day 4	Day 10	Day 2	Day 4	Day 10
Control	69	23	0	69	23	0	69	23	0
Cethromycin (6.25)	100	92	54	100	100	67	100	100	86–100
Cethromycin (12.5)	100	100	69	100	100	77	100	100	100
Erythromycin (37.5)							100	80	38
Erythromycin (50) ^a	100*	47*	27*	100*	100*	53*	100	100	50

^a *, Data are from one of our previous studies (3).

ratios close to 0 after a single 50-mg/kg erythromycin treatment and equal to 39 and 330 in sera and lungs, respectively, for cethromycin after a single oral dose of 25 mg/kg. Free T>MIC values were also longer for cethromycin than for erythromycin (Table 3). Our results are in keeping with a report from Mitten et al. (20), who found that cethromycin improved the survival of mice infected with resistant *S. pneumoniae* strains containing either the *ermB* gene or the *mefE* gene. This could be also expected from the sustained in vitro activity of cethromycin compared to erythromycin against the MLSB-resistant strain, P-6254, a result that is in accordance with those of several other investigators (15, 18, 24), who showed that cethromycin demonstrated high in vitro activity against a variety of resistant phenotypes, including the MLSB constitutive one. Cethromycin was found to be the most active agent against isolates of *S. pneumoniae* derived from community-acquired respiratory tract infections resistant to macrolides, with some quinolone-resistant strains, followed by telithromycin, azithromycin, clarithromycin, and erythromycin (27). It has been shown that cethromycin is active against all erythromycin-resistant strains, irrespective of the mode of resistance, *mef* or *ermB* mediated (31), or *ermAM* or efflux phenotypes (5) *ermB* and *mefA* (8, 14). This could be related to the fact that cethromycin binds tightly to ribosomes of a broad spectrum of resistant *S. pneumoniae* strains, including inducible and constitutive MLS, and *mef*-containing strains (7). In addition, Capobianco et al. (7) confirmed erythromycin's complete lack of affinity with methylated ribosomes and suggested that cethromycin had lower affinity than for wild-type ribosomes but was able to block at higher drug concentrations.

Cethromycin was also more effective than erythromycin

against a macrolide-susceptible strain despite a similar in vitro activity of both antibiotics, with 100% survival with a 12.5-mg/kg s.c. dose of cethromycin versus 50% with a 50-mg/kg s.c. dose of erythromycin. Cethromycin had a longer $t_{1/2}$ in sera and lungs than did erythromycin, in both uninfected controls and infected mice, regardless of the administration route. However, when PK and PD indexes were evaluated on free drug fractions (5.2% obtained after a 12.5-mg/kg dose, similar to the 6% reported by Kim et al. [16], and 11.5% after a 25-mg/kg dose for cethromycin, versus 80% for erythromycin), the serum AUC_{free}/MIC ratios were lower with cethromycin than with erythromycin. Similarly, the free T>MIC was shorter after a 12.5-mg/kg dose of cethromycin than after a 50-mg/kg dose of erythromycin (Table 3) despite a higher in vivo activity.

This higher efficacy of cethromycin against the susceptible strain might be explained by its structure and by its physicochemical properties. Capobianco et al. (7) have shown that cethromycin binds tightly to wild-type ribosomes and inhibits ribosomal functions. A macrolide-susceptible strain accumulated cethromycin more quickly than erythromycin. The more rapid accumulation of cethromycin compared to erythromycin in susceptible strains of *S. pneumoniae* was dictated by the tighter binding kinetics of the ketolide compared to the macrolide. Macrolides are more active at an alkaline pH and less active at an acidic pH (22). The chemical structure transformation of the 3-keto functional group, which is semisynthesized by the oxidation of the 3-OH group, confers a high degree of stability to ketolides in acidic environments (6). Fiese and Steffen (12) have shown, for example, that in acidic aqueous media erythromycin is rapidly degraded to products that possess little antimicrobial activity. It has also been shown

TABLE 5. Time course of bacterial clearance from lungs and blood of animals infected with the penicillin- and macrolide-resistant strain P-6254

Time postdosing ^a	Time p.i. ^b (h)	Mean bacterial count (CFU/ml) ^c ± SD after s.c. treatment with cethromycin at:			
		0 mg/kg	6.25 mg/kg	12.5 mg/kg	25 mg/kg
12 h after one Tt	18	5.4 ± 0.5 (3/3)	2.8 ± 0.1 (3/3)	2.2 ± 0.8 (3/3)	1.6 ± 0.9 (1/3)
12 h after two Tt	30	5.3 ± 0.4 (2/2)	2.7 ± 2.5 (3/3)	1.8 ± 1.2 (2/3)	3.0 ± 1.8 (2/3)
12 h after four Tt	54	>8.0 ^d	1.7 ± 1.1 (1/3)	1.2 ± 0.4 (2/3)	<1 ^e (3/3)
12 h after six Tt	78		<1 (0/3)	<1 (0/3)	<1 (0/3)

^a Tt, treatment(s) (the first treatment was given 6 h after bacterial challenge).

^b p.i., postinfection.

^c Bacterial counts in the lung are expressed as the log₁₀ CFU per ml of whole-lung homogenate; values are means ± the standard deviation ($n = 3$). Values in parentheses are the number of animals with positive blood cultures/the total number of animals.

^d All of the mice died.

^e Less than the limit of detection (1 log₁₀ CFU/ml) in all three animals.

that erythromycin penetrated and accumulated in human cells in tissue culture, including phagocytes, and rapidly egressed when the cells were incubated in antibiotic-free medium (19). The stability at an acidic pH may contribute to the better efficacy on a susceptible strain of the ketolide than the macrolide at the pulmonary infective sites with a low pH (4).

Our *in vivo* results showed that colonies from untreated controls and cethromycin-treated animals infected by P-4241 or P-6254 maintained the same antibiotic profile as the inoculated strain. This is in accordance with Niluis et al. (25), who showed that cethromycin at concentrations above the MIC did not select for mutants with reduced susceptibility from four strains and selected for mutants very infrequently (10^{-10} to 10^{-12} at $2\times$ MIC to $4\times$ MIC) from eight other strains.

In conclusion, our data show that cethromycin is highly effective in a mouse model of pneumonia induced by a macrolide-susceptible and a macrolide-resistant strain of *S. pneumoniae*, probably due to its *in vitro* activity and physiochemical properties.

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