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Pharmacokinetic and In Vivo Studies with Azithromycin (CP-62,993), a New Macrolide with an Extended Half-Life and Excellent Tissue Distribution

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Azithromycin (CP-62,993), a new acid-stable 15-membered-ring macrolide, was well absorbed following oral administration in mice, rats, dogs, and cynomolgus monkeys. This compound exhibited a uniformly long elimination half-life and was distributed exceptionally well into all tissues. This extravascular penetration of azithromycin was demonstrated by tissue/plasma area-under-the-curve ratios ranging from 13.6 to 137 compared with ratios for erythromycin of 3.1 to 11.6. The significance of these pharmacokinetic advantages of azithromycin over erythromycin was shown through efficacy in a series of animal infection models. Azithromycin was orally effective in treating middle ear infections induced in gerbils by transbulla challenges with amoxicillin-resistant *Haemophilus influenzae* or susceptible *Streptococcus pneumoniae*; erythromycin failed and cefaclor was only marginally active against the *H. influenzae* challenge. Azithromycin was equivalent to cefaclor and erythromycin against *Streptococcus pneumoniae*. In mouse models, the new macrolide was 10-fold more potent than erythromycin and four other antibiotics against an anaerobic infection produced by *Fusobacterium necrophorum*. Similarly, azithromycin was effective against established tissue infections induced by *Salmonella enteritidis* (liver and spleen) and *Staphylococcus aureus* (thigh muscle); erythromycin failed against both infections. The oral and subcutaneous activities of azithromycin, erythromycin, and cefaclor were similar against acute systemic infections produced by *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus viridans*, or *S. aureus*, whereas azithromycin was more potent than erythromycin and cefaclor against the intracellular pathogen *Listeria monocytogenes*. The pharmacokinetic advantage of azithromycin over erythromycin in half-life was clearly demonstrated in prophylactic treatment of an acute mouse model of *S. aureus* infection. These properties of azithromycin strongly support the further evaluation of this new macrolide for use in community-acquired infections of skin or soft tissue and respiratory diseases.

Limitations to the clinical usefulness of erythromycin have been its ineffectiveness against *Haemophilus influenzae* and *Neisseria gonorrhoeae* and its low, erratic levels in blood. Much of the incomplete and erratic absorption of erythromycin is due to its inactivation at the pH of the stomach (19). In spite of these limitations, erythromycin has been a clinically useful antibiotic for the treatment of respiratory, cutaneous, and genital tract infections caused by a variety of organisms, such as *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Legionella pneumophila*, and *Chlamydia* spp. (16, 17).

Azithromycin (CP-62,993; also designated XZ-450 [Pliva Pharmaceuticals, Zagreb, Yugoslavia]) is a new macrolide antibiotic that differs structurally from erythromycin by a methyl-substituted nitrogen at position 9a in the macrolide ring. This modification has produced profound alterations of in vitro spectrum and potency (14) and superior stability to an acid environment (pH 2.0) compared with erythromycin (E. F. Fiese and S. H. Steffen, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 229, 1987).

In this paper we describe the pharmacokinetics of azithromycin in mice, rats, dogs, and monkeys and its in vivo activities against clinically relevant middle ear infections induced by transbulla challenge with human clinical isolates of *Streptococcus pneumoniae* or amoxicillin-resistant *H. influenzae*, tissue models of infection produced by *Fusobacterium necrophorum*, *Salmonella enteritidis*, or *S. aureus*, and human clinical isolates of *Streptococcus*, *S. aureus*, and *Listeria monocytogenes* in acute systemic models of infection. The tissue and intracellular models of disease were specifically included to investigate the influence of the pharmacokinetic advantage of azithromycin over erythromycin.

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MATERIALS AND METHODS

Pharmacokinetic studies. Male and female outbred CD1 mice (body weight, about 25 g) and male outbred CD rats (70 to 90 g) were purchased from Charles River Breeding Laboratories, Inc., Kingston, R.I. Purebred male beagle dogs (10 to 12 kg) and female cynomolgus monkeys (2.5 to 4.0 kg) were obtained from colonies maintained at Pfizer, Inc.

Single oral doses of azithromycin and erythromycin were administered to fasted (16 h) mice, rats, dogs, and monkeys in studies designed to examine pharmacokinetic parameters in plasma (mice and rats) or serum (dogs and monkeys). Suspensions of antibiotics were prepared by homogenizing

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the powders in a standard diluent (7) containing Methocel 15 (0.5 g), Polysorbate 80 (1.0 g), carboxymethylcellulose (CMC 70 low) (10.0 g), sodium chloride (9.0 g), and water (984 ml). Dose volumes were 0.2 ml for mice, 0.5 ml for rats, 100 ml for dogs, and 10 ml for monkeys; water was available ad libitum. While comparative groups of mice and rats received either azithromycin or erythromycin, dogs and monkeys were given drugs in a crossover design. Separate groups of rats were orally dosed once with azithromycin or erythromycin for determinations of pharmacokinetic parameters in tissues. A study directed at examining levels in lung tissue was done with rats given azithromycin as a single oral dose ranging from 10 to 200 mg/kg.

Mouse and rat peripheral blood samples were obtained by retroorbital bleeding into heparinized capillary pipettes. Serial samples were taken at 0.25 to 30 h after oral dosing. Similarly, serial blood samples were acquired from dogs and monkeys via venipuncture of jugular or femoral veins, respectively, between 0.5 and 24 h after dosing. For tissue distribution studies, three rats, dosed with either azithromycin or erythromycin, were exsanguinated via cardiac puncture at 0.5 to 24 h after dosing; tissue and blood samples were taken at these times to generate tissue distribution parameters. Tissues were processed as described previously (7). Briefly, tissues were removed, weighed, and homogenized in 10 volumes of buffered saline in Potter-Elvehjem tissue grinders powered by an overhead stirrer. Resulting supernatants were stored at 4°C and assayed on the same day or kept frozen at −20°C until analyzed at a later date.

All plasma or serum and tissue samples were analyzed by a modified agar well diffusion bioassay procedure (15). Standard curves for azithromycin and erythromycin were prepared in the same fluid or tissue homogenate from control mice, rats, dogs, or monkeys according to the experiment. The inoculum of *Micrococcus luteus* ATCC 9341 was 1 ml of a 1:100 dilution of a standardized frozen culture per 100 ml of Neomycin assay agar (Becton Dickinson and Co.). Incubation was at 37°C for approximately 18 h. The sensitivity of the assay procedure for both azithromycin and erythromycin was 0.02 μg/ml for serum and plasma samples and 0.1 μg/ml for tissue samples. Assay variability was approximately 5%.

Plasma and serum pharmacokinetic parameters were calculated for individual animals over the entire sampling period, and subsequent mean values were determined by averaging the values for all the animals in a dose group. Means for tissue level determinations were calculated from data obtained from a different group of three animals per time point. Pharmacokinetic data are expressed in terms of means and standard errors. The data were evaluated for statistical significance by Student's *t* test.

Pharmacokinetic parameters were defined as follows. T_{max} corresponds to the time that the peak was observed. $t_{1/2}$ or half-life was determined from the elimination rate constant as calculated by linear regression analysis from T_{max} to the last time point (8). Area under the curve ($AUC_{0-\infty}$) corresponds to the AUC from zero time to infinity, as calculated by the trapezoidal rule through the last time point and the terminal area as determined from the ratio of the last concentration to the terminal rate constant.

Antibacterial agents. Azithromycin was prepared in the Pfizer Central Research Medicinal Chemistry Laboratories. Erythromycin A base was obtained in bulk from Upjohn Laboratories. Azithromycin and erythromycin were administered in their free-base forms. All other antimicrobial agents were obtained from their pharmaceutical manufacturers. In all studies that developed either 50% effective dose (ED_{50}) or 50% protective dose (PD_{50}) values, the dose range consisted of four different antibiotic concentrations in a twofold dilution series.

Microorganisms. *F. necrophorum* ATCC 27852 was obtained from T. D. Wilkins, Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg; *Salmonella enteritidis* NCTC 5694 was a gift of F. M. Collins, Trudeau Institute, Saranac Lake, N.Y. *S. aureus* ATCC 12384, previously deposited by Pfizer Inc., and *Streptococcus pyogenes* ATCC 21351 were obtained from the American Type Culture Collection, Rockville, Md. The other microorganisms used were clinical isolates obtained from hospitals in the eastern United States. The cultures were maintained at −70°C and subcultured two or three times before use in animal experiments.

Middle ear infection in gerbils. The anatomy and histology of the eustachian tube and middle ear of the Mongolian gerbil (*Meriones unguiculatus*) are quite similar to those of chinchillas (4), and the usefulness of the gerbil as an inbred model for otitis media was demonstrated by Fulghum et al. (9) and Chisholm et al. (D. R. Chisholm, A. M. Henderson, G. A. Leonard, R. K. Mentley, and J. V. Desiderio, 25th ICAAC, abstr. no. 587, 1985). Female gerbils (50 g) were infected by injecting 0.1 ml of inoculum prepared from 4- to 6-h broth cultures of *H. influenzae* 54131 or *Streptococcus pneumoniae* 02J025 directly into the left bulla. *H. influenzae* was grown in brain-heart infusion broth (BHI; Scott Laboratories, Inc.) supplemented with 4% Fildes enrichment and 2% cofactor (Quiger Laboratory) and diluted to 10^{-3} in this medium to yield a final inoculum size of 2×10^5 cells per gerbil. The *Streptococcus pneumoniae* inoculum was grown in BHI broth supplemented with 10% defibrinated sheep blood (Quiger) and diluted to 10^{-5} in BHI broth to yield a final inoculum size of about 5×10^2 cells per gerbil. Both inocula were prepared by incubation at 37°C in an atmosphere of 5% CO_2. Gerbils were treated orally (three times a day for *H. influenzae* or twice a day for *Streptococcus pneumoniae*; five gerbils per treatment group) with compound on days 1 and 2 postchallenge. Animals were sacrificed on day 3, and an ear washing sample was taken from each gerbil. Samples of aspirates were plated on blood agar for recovery of *Streptococcus pneumoniae* or BHI agar supplemented with Fildes enrichment and cofactor for recovery of *H. influenzae*. The plates were examined for growth after incubation in an atmosphere of 5% CO_2 at 37°C for 24 h. Treatment was considered effective if washings (1:100 dilution) from treated gerbils showed less than 10 CFU per plate. After four to five experiments were completed, data were averaged and an ED_{50} (expressed in milligrams per kilogram) was calculated by the method of Batson (1). In making this calculation, negative cultures per total animals treated at a given level of drug were employed.

Experimental anaerobic infection. This experimental anaerobic infection was originally described by Wilkins and Smith (18). *F. necrophorum* ATCC 27852 was grown in a prereduced chopped-meat medium (Scott) under strict anaerobic conditions. All sterile plastic disposable syringes, rubber stoppers, empty glass tubes, pipettes, etc., used to prepare the inoculum were degassed overnight in an anaerobic chamber (Coy Lab Products, Inc.). Male C3H/HeN mice (18 to 20 g) were injected intraperitoneally with 0.2 ml of an undiluted overnight culture (about 3.6×10^8 per mouse). Antibiotic treatments (10 mice per group) were given orally or subcutaneously at 22 and 28 h following challenge. Untreated challenged animals died 5 to 7 days postchallenge. After a holding period of 9 to 10 days, PD_{50}s

TABLE 1. Pharmacokinetic parameters of azithromycin and erythromycin after oral dosage to mice, rats, dogs, and monkeys[a]

Animal (no.)	Dose (mg/kg)	Compound	Peak concn in serum or plasma (μg/ml)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (μg · h/ml)
Mice (20)	50	Azithromycin	1.63 ± 0.15	1.45 ± 0.17	6.42 ± 0.85	10.0 ± 1.13
		Erythromycin	0.91 ± 0.11	0.95 ± 0.11	1.19 ± 0.12	1.53 ± 0.15
Rats (20)	50	Azithromycin	1.27 ± 0.14	1.95 ± 0.19	7.71 ± 0.84	9.52 ± 0.93
		Erythromycin	0.56 ± 0.08	1.71 ± 0.12	2.11 ± 0.29	1.57 ± 0.13
Dogs (10)	10	Azithromycin	1.35 ± 0.12	0.90 ± 0.15	21.0 ± 1.74	23.4 ± 2.47
		Erythromycin	2.45 ± 0.34	0.55 ± 0.05	1.46 ± 0.06	5.23 ± 0.85
Monkeys (4)	10	Azithromycin	0.43 ± 0.08	2.25 ± 0.63	8.68 ± 0.67	2.92 ± 0.24
		Erythromycin	0.17 ± 0.12	1.50 ± 0.50	0.63 ± 0.26	0.22 ± 0.13

[a] Values are means ± standard error.

were calculated by the method noted earlier for ED_{50}. The PD_{50}s for these and all studies described below were calculated with data from at least two experiments.

Oral challenge with *Salmonella enteritidis*. Challenge (*Salmonella enteritidis* NCTC 5694 grown overnight in BHI broth at 37°C and diluted 10^{-3} in BHI broth to yield an inoculum size of approximately 5×10^6 CFU in BHI broth) was given to male C3H/HeN mice (18 to 20 g) by gavage. Cyclophosphamide (Aldrich Chemical Co.; 200 mg/kg) was given subcutaneously in a single dose 2 h postchallenge. Treatments (10 mice per group) were given by gavage either prophylactically (24 h prechallenge), or therapeutically (early: 20, 26, and 48 h postchallenge; late: 100, 124, 130, 144, and 150 h postchallenge). Untreated challenged controls died on postchallenge day 7 to 11; PD_{50}s were calculated after animals were held for at least 11 days postchallenge.

Localized *S. aureus* thigh infection in mice. Mice (CF1; 11 to 13 g; both sexes) were challenged (1.8×10^7 CFU per mouse) intramuscularly with 0.2 ml of a 1:5 dilution of a log-phase culture of *S. aureus* ATCC 12384 grown in BHI broth at 37°C. This challenge, remaining localized, did not result in death of any of the animals used. Treatments (five mice per group) were first given orally at 18 h postchallenge and were continued twice a day on days 1, 2, and 3 postchallenge. On day 6 following challenge (3 days after the last treatment), mice were sacrificed and tissue was taken as reported previously (6). Tissue from a single animal was then homogenized (Stomacher 80; Tekmar Co.), and an appropriate volume of sterile saline was added to yield a final tissue dilution of 1:100 (grams per 100 ml). Dilutions were spread on the surface of mannitol-salt agar (Scott) and incubated at 37°C for 48 h. Counts were expressed as viable organisms per thigh.

Prophylactic effect on an acute systemic *S. aureus* infection. Antibiotics (100 mg/kg) were administered subcutaneously as a single treatment to groups of 10 mice (CD1; 11 to 15 g) at various times (0 to 24 h) before an intraperitoneal challenge (about 7×10^8 CFU per mouse) with *S. aureus* ATCC 12384 in 5% hog gastric mucin. After challenge, the mice were maintained for an additional 96 h; the percent surviving was recorded at that time.

Acute systemic infections. Most acute systemic infections were produced in CD1 mice (11 to 15 g) by intraperitoneal inoculation of cultures grown overnight in BHI broth, appropriately diluted in BHI broth, and suspended in 5% hog gastric mucin to yield from 1 to 10 100% lethal doses. *Streptococcus pyogenes* ATCC 21351 for challenge was prepared in BHI broth without addition of mucin. Mice (10 per group) were treated orally or subcutaneously, commencing 0.5 h after challenge, with subsequent treatments at 4 and 24 h. For *L. monocytogenes* 12A005 challenge, treatments were given at 0.5 and 4 h postchallenge, followed by twice-daily administration on days 2 and 3 following challenge. Percent survival was recorded after a 4-day observation period, and the PD_{50} was calculated.

RESULTS

Plasma and serum pharmacokinetics. The plasma and serum profiles of azithromycin and erythromycin, expressed as the relevant mean pharmacokinetic parameters following a single oral dose in mice, rats, dogs, and monkeys, are summarized in Table 1. Azithromycin exhibited a prolonged elimination $t_{1/2}$ in all species examined, ranging from 6.4 h in mice to 21.0 h in dogs. Measurable levels of drug were still present at 24 h postdose. The elimination of erythromycin was much more rapid, with $t_{1/2}$s of 1.2 and 1.5 h in mice and dogs, respectively. Peak levels for azithromycin were higher than for erythromycin except in dogs. Both azithromycin and erythromycin displayed a short lag in absorption, as measured by T_{max}, with erythromycin attaining peak concentrations slightly earlier than azithromycin. The AUC values for azithromycin ranged from 4.5- to 13-fold higher than those for erythromycin in the species studied.

Tissue distribution in rats. The tissue distribution profiles of azithromycin and erythromycin, expressed as the relevant mean pharmacokinetic parameters following a single oral dose of 50 mg/kg, are summarized in Table 2. These profiles were developed to provide insight into the observed prolonged half-lifes in plasma of azithromycin relative to erythromycin. The peak levels of azithromycin occurred later than those of erythromycin in all tissues examined. Both drugs were widely distributed to all tissues, but azithromycin achieved from 2- to 10-fold-higher peak levels than erythromycin. This difference was most notable in the kidney, spleen, and liver, where levels of azithromycin (17.5 to 73.4 μg/g) were from 12- to 50-fold higher than in plasma. The residence times for azithromycin in all tissues studied were clearly longer than those achieved with an equal dose of erythromycin. The extrapolated elimination half-life for azithromycin in kidney, spleen, and liver was 32, 37, and 12 h, respectively. These values were as much as 32-fold longer than those obtained for erythromycin, which was rapidly eliminated from tissues, paralleling its plasma pharmacokinetics. The tissue pharmacokinetic difference between azithromycin and erythromycin was more evident in a comparison of AUC values. The lowest AUC value for azithromycin was 165 μg · h/g (muscle); the highest for erythromycin was 19.5 μg · h/g (spleen). The AUC for azithromycin in spleen was 1661 μg · h/g. The ability of antibiotics to penetrate tissue is best expressed by the ratio of the AUC for the antibiotic in tissue to the AUC for serum or plasma (2);

TABLE 2. Tissue distribution of azithromycin and erythromycin in rats following an oral dose of 50 mg/kg[a]

Sample[b]	Compound	Peak concn (μg/ml or g)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (μg · h/ml or g)	AUC ratio (tissue/plasma)
Plasma	Azithromycin	1.49	1.33	7.54	12.10	
	Erythromycin	0.71	1.66	1.12	1.67	
Kidney	Azithromycin	17.50	3.33	32.40	773.00	63.90
	Erythromycin	4.47	1.66	1.01	9.02	5.40
Spleen	Azithromycin	44.60	4.00	36.90	1,661.00	137.00
	Erythromycin	4.00	1.00	1.81	19.50	11.60
Liver	Azithromycin	73.40	4.00	11.70	1,210.00	100.00
	Erythromycin	8.87	2.00	0.89	18.70	11.20
Lung	Azithromycin	18.20	4.00	16.00	372.00	30.80
	Erythromycin	3.93	1.50	1.08	9.34	5.59
Muscle	Azithromycin	6.27	4.00	18.30	165.00	13.60
	Erythromycin	3.20	2.00	0.60	5.15	3.08

[a] Mean of three determinations per compound.
[b] Plasma and tissue samples (three per period per compound) were taken at 0.5, 1, 2, 4, 6, and 24 h postdosing.

increasing values reflect greater tissue penetration. Although erythromycin and azithromycin had tissue AUC values that were larger than plasma values, azithromycin tissue/plasma exposure ratios ranged from 13.6 to 137, while ratios for erythromycin ranged from 3.1 to 11.6 for skeletal muscle and spleen tissues, respectively.

Because of the important therapeutic use of macrolides in respiratory disease, focus was placed on the lung in expanded studies that included varying the oral dose of azithromycin and extending the sampling period beyond 24 h. In contrast to erythromycin, azithromycin was shown to give high and sustained levels in lung (Fig. 1). In addition, the levels of azithromycin achieved in rat lung tissue were essentially linear for doses between 10 and 200 mg/kg, with measurable drug levels detected past 90 h even for the 10-mg/kg dose.

Middle ear infections. The gerbil model employed in these studies was designed to represent localized middle ear infections. These infections were produced at a site (bulla) that is difficult for drug penetration and therefore provided a system for rigorous evaluation of antibiotic performance.

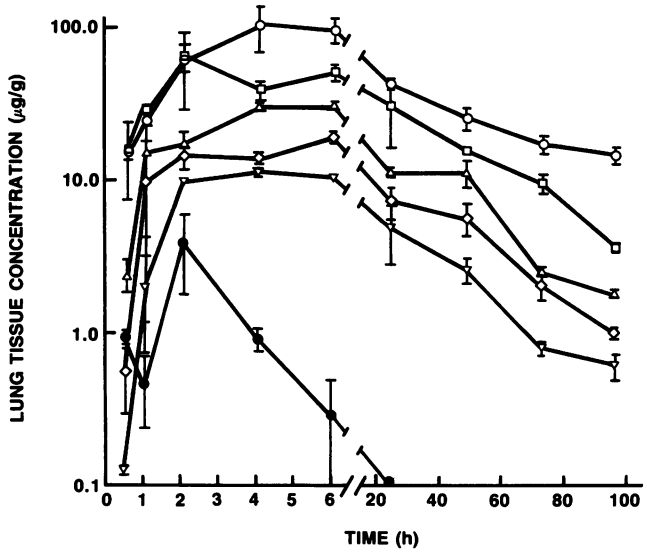

FIG. 1. Azithromycin and erythromycin concentrations in rat lung tissue (mean ± standard error) following single oral doses. Azithromycin concentrations following a 200-mg/kg (○), 100-mg/kg (□), 50-mg/kg (△), 20-mg/kg (◇), and 10-mg/kg (▽) oral dose; erythromycin concentrations following a 50-mg/kg (●) oral dose.

Orally administered azithromycin was effective (ED_{50}, 36.7 mg/kg) against the middle ear infection induced by amoxicillin-resistant *H. influenzae*; erythromycin and amoxicillin (negative control) failed (Table 3). Cefaclor was shown to have less activity than azithromycin and expressed a wide range of effectiveness from study to study (ED_{50}, 17.2 to >100 mg/kg, with an ED_{50} of >100 mg/kg in two of five studies). In contrast, azithromycin consistently expressed activity (ED_{50} range, 27.1 to 59.3 mg/kg). The consistent demonstrability of azithromycin effectiveness may be related to its stability in response to challenge size, while the inconsistent activity of cefaclor may be due to its relatively high MIC (12.5 μg/ml), resulting in greater instability to variations in challenge size, i.e., in vivo inoculum effect.

Azithromycin given orally was equivalent to erythromycin and cefaclor in the middle ear infection induced by *Streptococcus pneumoniae*. Amoxicillin was the most potent antibiotic against this infection.

Experimental anaerobic infection. Withholding the first treatment for 22 h postchallenge allowed a tissue infection to be established in this model of liver abscess produced by *F. necrophorum*. Azithromycin yielded an oral PD_{50} of 10.0 mg/kg against this infection, while erythromycin and amoxicillin failed (Table 4). Subcutaneously, azithromycin was effective (PD_{50}, 5.7 mg/kg) against this model of abscess, whereas erythromycin again failed at 100 mg/kg. Cefoxitin, although having an in vitro potency equivalent to azithromycin, failed in this treatment regimen.

TABLE 3. Oral activity of azithromycin in gerbil model of middle ear infection

Pathogen[a]	Compound	MIC (μg/ml)	ED_{50} (mg/kg)[b]
H. influenzae 54A131	Azithromycin	0.10	36.7 (±3.8)
	Erythromycin	1.56	>100
	Cefaclor	12.5	100 (±35)
	Amoxicillin	>100	>100
Streptococcus pneumoniae 02J025	Azithromycin	0.02	4.1 (±0.7)
	Erythromycin	0.01	6.8 (±2.0)
	Cefaclor	0.10	4.4 (±0.4)
	Amoxicillin	0.003	<1.56[c]

[a] Pfizer culture designations.
[b] Numbers in parentheses are 95% confidence limits.
[c] 100% effective at 1.56 mg/kg.

TABLE 4. Comparative activity of azithromycin and control antibiotics against an established *F. necrophorum* anaerobic infection in mice

Compound	MIC (μg/ml)	PD_{50} (mg/kg)[a]	
		Oral	Subcutaneous
Azithromycin	0.20	10.0 (±1.1)	5.7 (±0.7)
Amoxicillin	0.39	>100	ND[b]
Cephalexin	3.12	>100	ND
Erythromycin	3.12	>50	>100
Cefoxitin	0.20	ND	>100
Ampicillin	0.78	ND	>100

[a] Numbers in parentheses are 95% confidence limits.
[b] ND, Not done.

Oral challenge of immunosuppressed mice with *Salmonella enteritidis*. Azithromycin was the only antibiotic tested that consistently protected mice against *Salmonella enteritidis* infection (Table 5). Its oral PD_{50} was 7.6 and 8.8 mg/kg (early and late therapy, respectively) compared with >100 mg/kg for erythromycin or cefaclor. Azithromycin demonstrated a prophylactic effect when administered as much as 24 h prior to challenge with *Salmonella enteritidis*.

Localized *S. aureus* thigh infection in mice. The results of the *S. aureus* studies are shown in Table 6. A significant decrease (P=<.01) (99.9% reduction) of recoverable *S. aureus* was seen in the group treated with 100 mg of azithromycin per kg, but not with erythromycin at the same dose.

Effect of pretreatment with azithromycin or erythromycin on *S. aureus* infection in mice. The accumulated data presented in Table 7 show that azithromycin protected 60% of the mice when the single treatment at 100 mg/kg was given as much as 8 h prior to the initiation of a fatal *S. aureus* infection. In contrast, erythromycin at an equal dose protected <1% of the animals when given 8 h prior to challenge and only 48% when administered 0.5 h prior to infection.

Acute systemic infections. Except for the oral activity of cefaclor against *S. aureus*, the oral and subcutaneous activities of azithromycin, erythromycin, and cefaclor against these gram-positive cocci were similar (Table 8). In contrast, the in vitro potency of erythromycin against these organisms was slightly greater than that of azithromycin. Erythromycin and azithromycin were generally more potent than cefaclor in vitro. The oral activity of azithromycin (PD_{50}, 1.2 mg/kg) against *L. monocytogenes* was greater than that of erythromycin (PD_{50}, 18.4 mg/kg), although erythromycin exhibited greater in vitro potency. Parenteral dosing with cefazolin at doses as high as 100 mg/kg failed to protect mice from the *L. monocytogenes* challenge; cefazolin was very active in vitro against this organism (MIC, 0.39 μg/ml). This is consistent with the failure of cephalosporins in treating listeriosis in humans (11).

TABLE 5. Therapeutic and prophylactic oral efficacy of azithromycin against a *Salmonella enteritidis* challenge in immunosuppressed mice

Regimen	PD_{50} (mg/kg)[a]		
	Azithromycin[b]	Erythromycin	Cefaclor
Therapeutic[c]			
Early	7.6 (±1.1)	>100	>100
Late	8.8 (±2.2)	>100	>100
Prophylactic (24 h prechallenge)	44.9 (±8.9)	ND[d]	ND

[a] MICs of azithromycin, erythromycin, and cefaclor for *Salmonella enteritidis* were 0.78, 12.5, and 0.78 μg/ml, respectively.
[b] Numbers in parentheses are 95% confidence limits.
[c] Early, 20, 26, and 48 h postchallenge; late, 100, 124, 130, 144, and 150 h postchallenge.
[d] ND, Not done.

TABLE 6. Comparative oral activity of azithromycin and erythromycin against intramuscular *S. aureus* challenge in mice

Compound	Dose (mg/kg)	MIC (μg/ml)	Geometric mean[a] $\log_{10}$ organisms/thigh ± SD
Azithromycin	100	0.05	3.47 ± 1.65[b]
Erythromycin	100	0.01	5.69 ± 1.46
Untreated controls			6.47 ± 1.00

[a] Five mice per group.
[b] P = <0.01.

DISCUSSION

Azithromycin is a new macrolide antibiotic with a structural modification that confers greater gastric acid stability than erythromycin. The superior stability of azithromycin to gastric acid compared with erythromycin base is evidenced by a 10% loss of compound after 82 min at pH 2.0 compared with 7 s for erythromycin (E. F. Fiese and S. H. Steffen, 27th ICAAC, abstr. no. 229, 1987). Because of this improvement and the interesting in vitro spectrum of the new compound, comparative pharmacokinetic and in vivo efficacy studies of azithromycin and erythromycin were conducted.

Since there is no good correlation between the extent of oral absorption of macrolides in any one animal species and humans (5), several species were included in these studies. Azithromycin is well absorbed in all species; this is probably related to its increased acid stability. Azithromycin expressed higher plasma AUC values and a longer $t_{1/2}$ than erythromycin in all animal species examined; these plasma profiles are related to its good penetration into and slow elimination from tissues.

The compound was characterized as having up to 11-fold-higher levels in tissue than erythromycin and an extremely long elimination half-life from all tissues. The slow egress of azithromycin from the tissues produced AUC tissue/plasma ratios that were generally 10-fold higher than those with erythromycin. Azithromycin has been shown to have a clear pharmacokinetic advantage over erythromycin in its ability to achieve and maintain high levels in rat lung tissue; this is of particular interest for therapeutic application in respira-

TABLE 7. Effect of pretreatment with azithromycin or erythromycin on *S. aureus* infection in mice

Time of treatment[a] (h prechallenge)	% of mice alive[b]	
	Azithromycin	Erythromycin
0	87	83
0.5	87	48
1	73	38
2	62	16
4	60	0.8
8	60	0.03
24	27	0.03

[a] Subcutaneous treatment; single dose of 100 mg/kg.
[b] Average values of three individual experiments.

TABLE 8. Comparative activity of azithromycin, erythromycin, and cefaclor against acute systemic gram-positive experimental infections in mice

Challenge strain	Compound	MIC (μg/ml)	PD_{50} (mg/kg)[a]	
			Oral	Subcutaneous
Streptococcus pneumoniae O2J023[b]	Azithromycin	0.19	3.0 (±0.4)	1.2 (±0.3)
	Erythromycin	0.09	5.5 (±1.4)	1.4 (±0.4)
	Cefaclor	0.78	4.0 (±0.8)	12.7 (±0.6)
Streptococcus pyogenes ATCC 21351	Azithromycin	0.19	2.6 (±0.6)	0.5 (±0.1)
	Erythromycin	0.04	5.7 (±1.5)	0.6 (±0.1)
	Cefaclor	0.10	1.3 (±0.2)	0.9 (±0.2)
Viridans group streptococci (strain O2F014)	Azithromycin	0.19	4.8 (±1.6)	0.8 (±0.7)
	Erythromycin	0.09	14.3 (±2.7)	0.1 (±0.1)
	Cefaclor	1.56	2.8 (±0.4)	2.2 (±0.8)
S. aureus ATCC 12384	Azithromycin	0.05	71.0 (±6.6)	8.5 (±2.4)
	Erythromycin	0.01	100.0 (±8.9)	3.1 (±0.8)
	Cefaclor	3.12	1.2 (±0.3)	2.9 (±0.6)
L. monocytogenes 12A005	Azithromycin	0.19	1.2 (±1.1)	ND[c]
	Erythromycin	0.02	18.4 (±2.8)	ND
	Cefazolin	0.39	ND	>100

[a] Numbers in parentheses are 95% confidence limits.
[b] Pfizer culture designation.
[c] ND, Not done.

tory disease. Although the concentrations in lung were high, it is equally important that proportionality was observed after single doses of up to 200 mg/kg, and the rate of elimination of drug was essentially the same for all doses and subsequent levels achieved in lung tissue. A single oral dose of azithromycin, given at 10 mg/kg, yielded a level in lung tissue that remained above its MIC for 90% of isolates (14) of *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *H. influenzae*, and *Legionella pneumophila* for 48 to 72 h. The unusual predilection of azithromycin for extravascular foci and its extended tissue residence times clearly suggested that it would perform well compared with erythromycin against models of tissue-associated and other localized infections such as abscesses and infections of the middle ear, liver, spleen, lung, and muscle. Recently, an evaluation of azithromycin pharmacokinetics in humans confirmed its longer half-life compared with erythromycin and its good tissue penetration (R. M. Shephard, D. J. Weidler, D. C. Garg, P. O. Madsen, C. E. Cox, K. H. Chan, and C. D. Bluestone, 27th ICAAC, abstr. no. 239, 1987).

Erythromycin is not indicated for treatment of *H. influenzae* infections, and its lack of activity against this organism represents one of the major limitations to its clinical effectiveness. The findings of the present studies support the preceding report (14) that showed azithromycin to be active in vitro against *H. influenzae* and *Streptococcus pneumoniae*, the two most important etiological agents in cases of human otitis media (12). The oral effectiveness of azithromycin against the middle ear infection induced by an amoxicillin-resistant *H. influenzae* is of particular interest, since this type of resistant pathogen is an important clincial problem in otitis media.

The tissue levels of azithromycin must play an important role in its efficacy against experimental *H. influenzae* and *Streptococcus pneumoniae* infections. In addition to its application in otitis media, its high levels in tissue make it an attractive agent for potential use against respiratory diseases associated with these two pathogens. Its increased concentration in tissue and half-life following a single 50-mg/kg dose yield an AUC in rat lung that was 40 times that of erythromycin. Therefore, the level of azithromycin in lung tissue exceeds its MIC for 90% of isolates of *H. influenzae* (14) for 64 h, compared with 1 h for erythromycin.

Of the drugs evaluated, only azithromycin was active against the established anaerobic infection. This infection provided a formidable site for antimicrobial agents to enter and resolve microbiologically; in fact, some foci of infection were visible in untreated controls at 22 to 24 h after challenge. Because this infection was anaerobic, closed off from the circulatory system, high tissue levels of the antibiotic are critical for a therapeutic response. Thus, the activity of azithromycin in this model indicates that the high tissue levels obtained with this compound are available for inhibiting growth of the anaerobic pathogen. Ampicillin and amoxicillin, both having in vitro potencies similar to that of azithromycin against *F. necrophorum*, were not efficacious in this model, demonstrating the difficulty of achieving and maintaining effective antimicrobial agent levels at this site. An earlier and more frequent or prolonged treatment regimen with these agents, but not erythromycin, would probably result in successful therapy. In fact, Wilkins and Smith (18) demonstrated the effectiveness of benzylpenicillin against this infection model by considerably extending the treatment regimen. The pharmacokinetics of the standard agents included in these studies are not adequate to compete with azithromycin within the framework of the regimen used, i.e., one that demanded attainment of high and sustained tissue levels for success.

The *Fusobacterium* anaerobic abscess model was designed to demonstrate the importance of antibiotic levels in tissue in the successful resolution of this infection site and did not focus on the broad application of the compound in anaerobic infections. However, azithromycin is weakly to moderately active against *Bacteroides fragilis* (14), clinically the most important human pathogenic anaerobe. When the tissue levels achieved by this compound are considered, this in vitro activity may translate into efficacy against a variety of anaerobic infections, including those caused by *B. fragilis*. Studies to assess the potential of this drug for use in serious anaerobic infection are planned, with models of self-resolving disease.

Salmonella enteritidis is an intracellular pathogen which, when given by gavage via the oral route, produces a slowly developing infection by initially infiltrating the Peyer's patches and then progressing to the liver and spleen by 3 to 5 days (3). Uncontrolled growth in these tissues yields bacteremia on day 6 or 7 postchallenge. Mortality usually follows shortly after detection of organisms in the blood. The early therapeutic dose regimen (20, 26, and 48 h postchallenge) used against the *Salmonella enteritidis* infection focused on control of infection within the Peyer's patches (3). Control of this phase of disease is dependent on effective levels in serum and tissue. Azithromycin was efficacious against this stage of infection while cefaclor failed, even though these two compounds have similar in vitro potency. Erythromycin failed, as expected with its poor in vitro potency.

Azithromycin was also effective in the regimen with late (first treatment at 100 h postchallenge) therapy. This is important because the disease was well established as an intracellular infection in the liver and spleen when the regimen was initiated. The efficacy of azithromycin at this stage of the infection is probably due to high and sustained tissue levels along with potent activity (MIC, 0.78 μg/ml against this strain of *Salmonella*). Additionally, the efficacy seen with late administration of azithromycin indicates that the azithromycin in the tissues is available to inhibit the

organism even when the pathogen is intracellular. Cefaclor and erythromycin failed because of poor in vitro potency or tissue pharmacokinetics. The long half-life of azithromycin in serum (seven times that of erythromycin) and tissues (13 and 20 times that of erythromycin in rat liver and spleen, respectively) is reflected in the prophylactic effectiveness of the compound against this *Salmonella* infection. This is the first macrolide to be reported as being effective in vivo against a lethal *Salmonella* infection.

Since the MIC of erythromycin (0.01 μg/ml) for the *S. aureus* used in the intramuscular infection is lower than that of azithromycin (MIC, 0.05 μg/ml), one might expect erythromycin to be more effective in vivo. However, as observed in all other models of tissue infection reported here, azithromycin was effective and outperformed erythromycin. This is in contrast to the indistinguishable PD_{50} values developed by these compounds when evaluated against this organism in an acute infection model. Again, the effectiveness of azithromycin can presumably be attributed to its tissue pharmacokinetics. The results of this particular study represent an important demonstration of the efficacy of azithromycin, since *S. aureus* infections commonly occur in skin and soft tissues.

Kawaler and Hof (11), in their recent discussion of experimental listeriosis, noted that the low efficacy of antibiotic therapy against this disease in humans can be due to the common occurrence of the disease in immunosuppressed hosts or in patients with debilitating disease and the ability of listeriae to multiply within phagocytes, in which it is difficult to achieve inhibitory concentrations of antibiotic. The pharmacokinetic studies reported here have shown azithromycin to have excellent penetrability into a wide variety of animal tissues. The potent in vivo activity against *L. monocytogenes* after oral administration of azithromycin is consistent with the notion that high tissue and phagocyte penetration by azithromycin results in inhibition of listeriae and subsequent protection from lethal effects. Although no determinations were made of the intracellular concentration of azithromycin, it is assumed that it will be increased over extracellular concentrations, based on data for macrolides such as erythromycin (10, 13).

All of the in vivo data presented here extend the attractive properties of azithromycin, underscore the importance of antibiotic levels in tissue in the control of disease, and strongly support the further evaluation of this new macrolide for use in community-acquired otitis media and skin, soft tissue, and respiratory diseases.

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