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Spectrum and Mode of Action of Azithromycin (CP-62,993), a New 15-Membered-Ring Macrolide with Improved Potency against Gram-Negative Organisms

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The macrolide antibiotic azithromycin (CP-62,993; 9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A; also designated XZ-450 [Pliva Pharmaceuticals, Zagreb, Yugoslavia]) showed a significant improvement in potency against gram-negative organisms compared with erythromycin while retaining the classic erythromycin spectrum. It was up to four times more potent than erythromycin against *Haemophilus influenzae* and *Neisseria gonorrhoeae* and twofold more potent against *Branhamella catarrhalis*, *Campylobacter* species, and *Legionella* species. It had activity similar to that of erythromycin against *Chlamydia* spp. Azithromycin was significantly more potent versus many genera of the family *Enterobacteriaceae*; its MIC for 90% of strains of *Escherichia*, *Salmonella*, *Shigella*, and *Yersinia* was ≤4 μg/ml, compared with 16 to 128 μg/ml for erythromycin. Azithromycin inhibited the majority of gram-positive organisms at ≤1 μg/ml. It displayed cross-resistance to erythromycin-resistant *Staphylococcus* and *Streptococcus* isolates. It had moderate activity against *Bacteroides fragilis* and was comparable to erythromycin against other anaerobic species. Azithromycin also demonstrated improved bactericidal activity in comparison with erythromycin. The mechanism of action of azithromycin was similar to that of erythromycin since azithromycin competed effectively for [^{14}C]erythromycin ribosome-binding sites.

Erythromycin has been regarded for many years as possessing a good spectrum of activity and safety record for the treatment of respiratory, skin, and soft tissue infections in both adults and children. Recent developments have tended to reinforce the importance of this antibiotic, as erythromycin is now the primary or secondary therapeutic agent for four prominent infections in humans: Legionnaires disease, *Mycoplasma* pneumonia, *Campylobacter* diarrhea, and chlamydial urethritis. However, the potential of erythromycin as a general-use oral antibiotic is limited by its modest potency against *Haemophilus influenzae* and *Neisseria gonorrhoeae* and by a low and erratic level in blood following oral administration. More recently, novel formulations or esters of erythromycin have been introduced to improve its pharmacokinetic properties. Each of these has incremental advantages, but none provides the kinetic improvements sufficient to completely incorporate *H. influenzae* and *N. gonorrhoeae* into the erythromycin spectrum.

Our research in this area has been aimed at identifying novel macrolide antibiotics with in vitro potency and pharmacokinetic properties that would incorporate activity against *H. influenzae* into the macrolide spectrum and allow for total lower doses. This paper reports the microbiological and biochemical properties of azithromycin (CP-62,993; also designated XZ-450 [Pliva Pharmaceuticals, Zagreb, Yugoslavia]), which differs from erythromycin chemically by a methyl-substituted nitrogen in the macrolide ring (Fig. 1). This difference produces improvements in spectrum and potency compared with erythromycin.

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MATERIALS AND METHODS

Antibiotics, microorganisms, and chemicals. The macrolide antibiotic azithromycin (9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A) and erythromycin base were prepared in the Pfizer Central Research Medicinal Chemistry Laboratories. Ampicillin, amoxicillin, cephalexin, cefaclor, and josamycin were commercial oral capsules. All antibiotics were corrected to 100% purity, sterilized in 0.25 ml of 100% ethanol, and diluted with sterile phosphate buffer (pH 6.5).

Microorganisms were recent clinical isolates obtained from hospitals in the United States. Beta-lactamase-producing (disk test, Marion Scientific) strains of *H. influenzae*, *Branhamella catarrhalis*, and *N. gonorrhoeae* were included.

Tris base, Cleland reagent, β-mercaptoethanol, lysostaphin, phosphoenolpyruvate, folinic acid, folic acid, *N*-formyl-L-methionine, GTP, ATP, and phenylmethylsulfonyl fluoride were from Sigma Chemical Co.; fMet-tRNA and tRNA (from *Escherichia coli* MRE600) were purchased from Boehringer Mannheim; EDTA K^+-Mg^{2+} salt came from Pfaltz and Bauer Inc.; lysozyme was a product of Worthington Biochemical Corp.; sodium dodecyl sulfate was from Aldrich Chemical Co., Inc.; pyruvate kinase was from Calbiochem; L-^{3}H-amino acid mix and [^{14}C]erythromycin (54 mCi/mmol) were obtained from New England Nuclear Corp.; and L-amino acids were from ICN Pharmaceuticals Inc. Iodouridine was from Smith, Kline and French Laboratories.

MIC determinations. The basal medium used for gram-positive and fastidious gram-negative species was brain-heart infusion (BHI) agar enriched as follows: 5% bovine

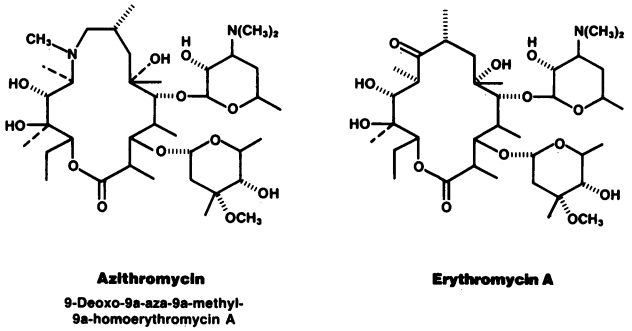

FIG. 1. Structures of azithromycin and erythromycin.

serum for *Streptococcus pneumoniae*, 1% hemoglobin (Difco Laboratories) for *B. catarrhalis*, and 5% Fildes enrichment and 2% cofactor (Quiger Lab) for *H. influenzae*. *N. gonorrhoeae* was grown on GC agar base enriched with 1% hemoglobin and 0.5% cofactor. *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *H. influenzae*, and *N. gonorrhoeae* were incubated in 3% CO_2. *Campylobacter* species were grown on tryptose agar enriched with 5% sheep blood; incubations were in GasPak jars (BBL Microbiology Laboratories) equipped with CampyPak generators (BBL). Anaerobes were grown in enriched BHI broth (10), and MICs were determined on Wilkins-Chalgren agar (Difco) and incubated under an 80% N_2–10% CO_2–10% H_2 gas mixture (O_2 free) in an anaerobic chamber. Agar dilution MICs were determined by the method of Ericsson and Sherris (6) by using the multiple inoculator described by Steers et al. (17). Cultures grown overnight in appropriately enriched BHI broth to $\geq 10^9$ cells per ml were diluted 100-fold in BHI broth; thus, approximately 10,000 to 20,000 cells of each strain were placed on the agar surface. Incubation was for 18 to 24 h at 37°C. Single colonies were disregarded in MIC determinations. The effect on potency of 95% fresh, pooled human serum was determined exactly as described by Bannatyne and Jackowski (2).

For genera of the family *Enterobacteriaceae* and other gram-negative species, MICs were determined by a broth microdilution technique. Twofold dilutions were done in Mueller-Hinton broth. The sterile medium containing the serially diluted antibiotics was dispensed into 96-well trays (0.2 ml per well) with the MIC-2000 dispenser (Dynatech). Trays were frozen at −70°C, and on the day of a test, the trays were put in the refrigerator (4°C) for 2 h and then held at room temperature to finish thawing. Test organisms were transferred three successive times in Mueller-Hinton broth and incubated for ~18 h at 37°C. A portion was plated and checked for purity. The last transfer amount was placed on the 37°C shaker to aerate the cultures and ensure good growth. After the last transfer, cultures were diluted 1:100 in Mueller-Hinton broth and placed in the MIC-2000 inoculator for delivery to the trays (final inoculum was ~10^6 CFU/ml). Following 18 h of incubation at 37°C, the MICs were read as the lowest dilution of antibiotic allowing no visible growth.

MICs against *Chlamydia trachomatis* were determined in McCoy monolayers. The monolayers were grown in the presence of iodouridine (20 μg/ml) for 4 days and washed with Hanks balanced salt solution. Then they were infected with the organism (~75 inclusion bodies per field) for 2 h before serial twofold dilutions of a drug were added to cells. Incubation was done at 37°C for 48 h in 5% CO_2. The monolayers were then washed and stained with iodine, and the cytoplasmic inclusion bodies were counted and compared with those in infected, nontreated controls.

Tissue cultures of human lung monolayers (MRC-5) were grown in microtiter plates in EMEM+ (Eagle minimum essential medium supplemented with fetal bovine serum [10%, vol/vol], 2 mM L-glutamine, and 1.5% $NaHCO_3$). They were washed in Hanks balanced salt solution and incubated with *Legionella pneumophila* in EMEM+ for 2 h at 37°C. The dilution of *L. pneumophila* used had previously been shown to result in complete lysis of the lung cells in 5 to 7 days. The infected cells were washed twice with Hanks balanced salt solution, followed by addition of 0.2 ml of EMEM+ containing the appropriate dilution of antibiotic. Cultures were then incubated for 1 week at 37°C in 5% CO_2 and examined microscopically each day for microbial growth. The MIC was defined as the lowest concentration of drug preventing cytopathogenic effect.

Determination of bactericidal activity. MBCs were obtained by first determining an MIC in microtiter trays containing 0.2 ml of BHI broth per well (enriched as described for *H. influenzae*). The inoculum of clinical isolates grown overnight in BHI broth (enriched for *H. influenzae*) was ~10^6 CFU/ml; incubation was at 37°C for 18 h. The MBC was determined by removing 0.01 ml from each well, streaking it on 10 ml of agar (1,000-fold dilution of residual antibiotic), and incubating overnight. The lowest concentration of antibiotic giving ≤5 colonies was recorded as the MBC. Thus, there was >99.9% death of the initial inoculum at the MBC.

The killing kinetics were determined in BHI broth with shaking and periodic plating of samples on agar as described previously (15); the agar plates (0.1 ml plated per 18 ml of agar) were incubated for 48 h before organisms were counted. Enriched medium was used for *H. influenzae*, and 5% sheep blood was added to the agar for *Streptococcus pyogenes*.

Preparation of *Staphylococcus aureus* S-30 cell free extract, S-148 enzyme, S-148 initiation factors, and ribosomes. A cell-free protein-synthesizing system derived from clinical isolates of *S. aureus* with natural mRNA was used. Since we are unaware of published reports of such a system, a detailed description follows. *S. aureus* strains 01A084 (erythromycin susceptible) and 01A038 (erythromycin resistant) were grown to an A_{540} of 1.0 (Gilford spectrophotometer) in BHI supplemented with 150 mg of folic acid per liter at 37°C in an incubator-shaker. Cells were recovered by centrifugation at 10,800 × *g* and washed twice by centrifugation at 12,000 × *g*. The first washing was with buffer 1 (0.01 M Tris hydrochloride, pH 7.8, 0.01 M $MgCl_2$, 1.0 M KCl, 5 mM EDTA K^+ Mg^{2+} salt, 0.01 M β-mercaptoethanol, 0.001 M Cleland reagent, and 10% [vol/vol] glycerol), and the second with buffer 2 (similar to buffer 1 but with 0.05 instead of 1.0 M KCl). The washed cells were suspended in buffer 3 (same as buffer 1 but with 0.06 M NH_4Cl instead of 1.0 M KCl), with 3.5 mM phenylmethylsulfonyl fluoride added to inhibit proteases. Lysozyme and lysostaphin were added to the cell suspension to give a final concentration of 8 mg/ml and 170 μg/ml, respectively. Next the suspension was frozen and thawed three times and incubated at 37°C for 15 min. Finally, the cells were disrupted with a French press and centrifuged at 17,000 × *g* for 15 min. The pellet was discarded, and the supernatant was centrifuged at 30,000 × *g* for 0.5 h. The supernatant (S-30) was dialyzed for 6 h at 4°C against a 1,000-fold excess of buffer 3.

The S-30 was centrifuged at 148,000 × *g* for 2 h at 0°C to separate the ribosomes from supernatant enzyme. The S-148

enzyme, containing the elongation factors, was stored at −70°C. The ribosomes were suspended in buffer 4 (same as buffer 3 but with 1.0 instead of 0.06 M NH_4Cl), mixed for 1 h in an ice bath, and centrifuged at 148,000 × *g* for 3 h at 0°C. The pelleted high-salt-washed ribosomes and supernatant were saved. Next, finely ground $(NH_4)_2SO_4$ was added to the supernatant to a 75% concentration with stirring for 1 h at 4°C. This was centrifuged at 20,000 × *g* for 15 min at 4°C; the supernatant was discarded. The pellet was suspended in buffer 5 (0.01 M Tris hydrochloride, pH 7.8, 0.005 M EDTA K^+ Mg^{2+} salt, 0.005 M β-mercaptoethanol, 0.001 M Clelands reagent, 10% [vol/vol] glycerol, and 3.5 mM phenylmethylsulfonyl fluoride) and dialyzed for 6 h against a 1,000-fold excess of buffer 5 at 4°C. This material, the S-148 initiation factors, was stored at −70°C.

The washed ribosomes were suspended in buffer 3, mixed for 1 h in an ice bath, and centrifuged at 148,000 × *g* for 3 h at 0°C. The ribosomes were suspended in buffer 3 and stored at −70°C.

Preparation of *E. coli* S-192 enzyme. *E. coli* MRE600 (ATCC 29417) was grown in the following modified M9 medium. One liter of tap water contained KH_2PO_4 (3.0 g), NaCl (0.5 g), NH_4Cl (1.0 g), Na_2HPO_4 (6.0 g), $MgSO_4$ (0.2 g), yeast extract (Difco; 50 mg), dextrose (4 g), and monosodium glutamate (2 g). Recovery of the cells and preparation of S-30 cell extract were accomplished by the same methods given for *S. aureus* except that no lysozyme, lysostaphin, or freezing and thawing was used. The S-30 was centrifuged at 192,000 × *g* for 3 h to give supernatant S-192 enzyme containing elongation factors.

Preparation of mRNA. *Streptococcus faecalis* ATCC 8043 was grown in the same manner as *S. aureus*. Harvested cells were washed and suspended in buffer D (0.01 M Tris hydrochloride, pH 7.6, 0.01 M NaCl, 0.005 M $MgCl_2$). Lysozyme and lysostaphin were added to the cell suspension to concentrations of 8 mg/ml and 170 μg/ml, respectively. The suspension was frozen and thawed once and incubated for 15 min at 37°C; sodium dodecyl sulfate was added to a 1% concentration, and the suspension was incubated again at 37°C until a decrease in opacity was evident. Next, sodium acetate buffer, pH 5.1, was added to 0.1 M and mixed with an equal volume of water-saturated phenol. Then the mixture was shaken vigorously in a 60°C water bath for 3 min. The phases were separated by centrifugation at 3,000 × *g* for 5 min, followed by removal of the aqueous layer and extraction with phenol again at 37°C. After removal of the aqueous layer, NaCl was added to a concentration of 1 M and mixed, and then mRNA was precipitated with 2 volumes of cold ethanol. This solution was held at −20°C overnight and centrifuged at 40,000 × *g* for 20 min. The precipitate was washed twice with 75% ethanol–25% 0.015 M NaCl solution, then dissolved in water, and stored at −70°C. The mRNA was heat treated before addition to the reaction mix; sodium acetate buffer, pH 5.1, was added to the mRNA solution to a concentration of 0.001 M, and the solution was then heated for 3 min in a 60°C water bath.

Protein synthesis assay. The S-192 enzyme (40 μg of protein) with 148-S ribosomes (90 μg of protein) in 0.1 M Tris hydrochloride (pH 7.8)–0.04 M NH_4Cl–8 mM $MgCl_2$–3.0 mM β-mercaptoethanol–1.0 mM Cleland reagent–0.2 mM EDTA K^+ Mg^{2+} salt was incubated for 15 min at 37°C in a volume of 24 μl. This preincubation mix was placed in a 4°C environment, and the following were added to give the indicated final concentrations: 0.1 M Tris hydrochloride (pH 7.8), 0.04 M NH_4Cl, 10 mM $MgCl_2$, 3 mM β-mercaptoethanol, 0.06 mM Cleland reagent, 0.2 mM EDTA K^+ Mg^{2+} salt, 0.3 mM GTP, 3.0 mM ATP, 50 mM phosphoenolpyruvate, 15 IU of pyruvate kinase, 2.0 μCi of 15 L-^{3}H-amino acids, and 100 μmol each of six L-amino acids (asparagine, cysteine, cystine, glutamic acid, methionine, and tryptophan), 0.005 U of fMET-tRNA, 0.025 A_{260} units of tRNA (deacylated), 0.8 A_{260} units of S-148 initiation factors, 2.3 mM *N*-formyl-L-methionine, 0.2 mM folinic acid, and 8 A_{260} units of heat-treated mRNA. Antibiotics were added where appropriate. The final volume of the reaction mix was 50 μl, and the reaction was initiated by placing the mix in a 37°C water bath. After 30 min, the reaction was terminated by addition of 3 ml of 5% trichloroacetic acid, and the reaction mix was heated for 5 min at 95°C and cooled on the laboratory bench before being placed in an ice bath for 1 h. Filters (0.8 μm pore size; Millipore Corp.) were prewashed with 3 ml of 5% trichloroacetic acid containing 1 g of Casamino Acids (Difco) per 100 ml. The precipitated reaction mix was poured through the prewashed filters and washed three times with 3 ml of 5% cold trichloroacetic acid. Filters were removed and allowed to air dry at room temperature for 1 h before being placed in scintillation vials. The extent of amino acid incorporation was determined by measuring the radioactivity remaining on the filters, which was 9,847 and 13,782 cpm for susceptible and resistant ribosomes, respectively, in the absence of inhibitor.

Ribosome-binding assay. The ability of macrolide antibiotics to inhibit [^{14}C]erythromycin binding to susceptible *S. aureus* high-salt-washed ribosomes was determined as described by Pestka (14). The complete 50-μl reaction mix contained 10 mM Tris hydrochloride (pH 7.2), 4 mM $MgCl_2$, 0.1 M KCl, 10 mM NH_4Cl, 5.0 A_{260} units of ribosomes, and 1.5 μM [^{14}C]erythromycin. Incubation was at 26°C for 20 min. The reaction was terminated by placing the reaction mix in an ice bath and adding 3 ml of cold reaction mix buffer; this was filtered through a 0.45-μm filter and counted as described by Pestka (14). Total erythromycin binding with no inhibitor was 4,105 cpm.

RESULTS

Susceptibility studies. The comparative MICs of azithromycin, erythromycin, and ampicillin against fastidious gram-negative genera are presented in Table 1. The azithromycin MIC for 90% of strains (MIC_{90}) was ≤1.56 μg/ml for all the genera tested. Azithromycin was four times more potent than erythromycin against *H. influenzae*, *N. gonorrhoeae*, *Campylobacter* spp., and *Pasteurella multocida* and was twice as active as erythromycin against penicillinase-producing *N. gonorrhoeae* and *B. catarrhalis*. The ampicillin (or amoxicillin) data show that many *H. influenzae* and *B. catarrhalis* isolates were also penicillinase producers.

Azithromycin was also active when tested against strains grown intracellularly in tissue culture. It was twice as potent as erythromycin against the five *L. pneumophila* strains tested and had good activity against the single strain of *C. trachomatis* included in these studies (Table 2).

Azithromycin demonstrated superior potency against all the *Enterobacteriaceae* genera and other aerobic gram-negative genera in comparison with erythromycin (Table 3), except when both drugs were inactive (e.g., against *Proteus mirabilis*). Against many species, azithromycin had equal or greater potency than cephalexin or ampicillin (*E. coli*, *Salmonella enteritidis*, *Shigella sonnei*, *Shigella flexneri*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Citrobacter diversus*). Azithromycin demonstrated activity (MIC_{90}, ≤4.0 μg/ml) against organisms that

TABLE 1. Susceptibility of fastidious gram-negative organisms to azithromycin

Organism (no. of strains)	% of strains inhibited	MIC (μg/ml)		
		Azithromycin	Erythromycin	Ampicillin
Haemophilus influenzae (70)	50	0.39	1.56	0.39
	90	0.78	3.12	>50
Branhamella catarrhalis (17)	50	≤0.015	0.03	0.25[a]
	90	0.03	0.06	4.0
Neisseria gonorrhoeae (11)	50	≤0.025	0.05	ND[b]
	90	0.05	0.39	
Penicillinase-producing *N. gonorrhoeae* (13)	50	0.062	0.125	>16
	90	0.125	0.25	
Campylobacter sp. (10)	50	0.25	0.5	2
	90	0.5	2	16
Pasteurella multocida (3)	100	0.10	1.56	0.10

[a] Amoxicillin, not ampicillin.
[b] ND, Not done.

cause gastrointestinal diarrheas, such as *E. coli*, *Salmonella enteritidis*, *Shigella sonnei*, *Shigella flexneri*, *Yersinia enterocolitica* (Table 3), and *Campylobacter* spp. (Table 1). Azithromycin was active (MIC, 4 μg/ml) against nine strains of *Salmonella enteritidis* and *Shigella flexneri* resistant to ampicillin and/or tetracycline. Some additional strains of *E. coli*, *Salmonella enteritidis*, and *Shigella* (total of 10) were tested at 10-fold higher and lower inoculum sizes (10^5 and 10^7 CFU/ml). Almost no inoculum effect was observed with azithromycin and erythromycin, while the average MIC of ampicillin increased by one dilution. Azithromycin was active against *Acinetobacter calcoaceticus* (MIC_{90}, 4 μg/ml), but not *Pseudomonas aeruginosa* (Table 3).

Azithromycin, in general, demonstrated about one-fourth to one-half the potency of erythromycin versus gram-positive species (Table 4). Thus, the MIC_{90} of azithromycin against erythromycin-susceptible strains of *S. aureus* and *Staphylococcus epidermidis* was ≤1.6 μg/ml (Table 4). Azithromycin and erythromycin both showed potent activity against *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Streptococcus agalactiae* strains. Azithromycin had activity similar to erythromycin against erythromycin-resistant gram-positive isolates, including *S. aureus*, *S. epidermidis*, *Streptococcus pyogenes*, and *Streptococcus faecalis*.

Using the membrane and pad method (2), we found that azithromycin and erythromycin were more potent against *S. aureus* Oxford (ATCC 9144) in the presence of 95% human serum than in normal broth medium; the MICs decreased, respectively, from 0.78 and 0.39 μg/ml to 0.025 and 0.05 μg/ml. Thus, in the presence of serum, azithromycin was more potent than erythromycin against *S. aureus* (this result was repeated with a second clinical strain). The MIC of the highly serum bound ceftriaxone increased from 0.5 to 15.6 μg/ml; this result agrees with that of Bannatyne and Jackowski (2). Enhancement of erythromycin activity by 50% heat-inactivated serum has been reported previously (7).

TABLE 2. Comparative activity of azithromycin and erythromycin against species grown in tissue culture

Organism and strain	MIC (μg/ml)	
	Azithromycin	Erythromycin
Legionella pneumophila[a]		
Philadelphia/1	0.5	1.0
Knoxville/1	0.5	1.0
Togus/2	0.5	1.0
Bloomington/3	0.25	1.0
Los Angeles/4	1.0	2.0
Chlamydia trachomatis[b]	0.025	0.0125

[a] Cultures were obtained from the Centers for Disease Control, Atlanta, Ga.
[b] Mean of three determinations.

While azithromycin and erythromycin showed similar activity against a wide variety of anaerobic genera (Table 5), both were considerably more active (MIC_{90}, 1.56 μg/ml [erythromycin] and 6.25 μg/ml [azithromycin]) than ampicillin (MIC_{90}, 50 μg/ml) or cephalexin (MIC_{90}, >200 μg/ml) against *Bacteroides fragilis*, probably because many of the *Bacteroides fragilis* isolates were beta-lactamase producers. Except for the poor activity of cefaclor against *Clostridium difficile* (the poor activity of cephalosporins versus *Clostridium difficile* has been reported previously [8]) and the macrolides against *Eubacterium limosum*, the macrolides and beta-lactams were generally active (MIC_{90}, ≤3.12 μg/ml) against anaerobic gram-positive bacteria.

Bactericidal activity. Overall, azithromycin demonstrated greater bactericidal activity than erythromycin, especially in MBC determinations. The MBC of azithromycin was within one dilution of its MIC for *Streptococcus pyogenes* and *H. influenzae* (Table 6), while the MBC of erythromycin was within two dilutions of its MIC for *Streptococcus pyogenes* and four dilutions for *H. influenzae*. Neither macrolide was bactericidal for *S. aureus* (MBC equal to or one dilution higher than the MIC); however, in this study dicloxicillin and cefaclor were also not bactericidal (Table 6). In a killing-kinetics experiment, azithromycin at four times its MIC killed >99.9% of the initial inoculum of a susceptible *S. aureus* strain within 24 h (Fig. 2a); erythromycin and ampicillin, at four times the respective MIC for the strain, only killed 99% of the inoculum. Both azithromycin and erythromycin at four times the MIC killed >99.9% of a *Streptococcus pyogenes* culture within 8 h; at their MIC, 24 h was required for 99.9% killing (Fig. 2b). Azithromycin at its MIC and erythromycin at or four times its MIC were initially bacteriostatic against *H. influenzae*, but after 24 h >99.9% of the culture was killed (Fig. 2c). At four times its MIC, azithromycin reduced the CFU by 90% in 8 h; no CFU were detected at 24 h. Against a clinical *E. coli* strain, azithromycin killed the culture within 24 h at four times its MIC (12.5 μg/ml); neither azithromycin or erythromycin was bactericidal at the MIC (Fig. 2d).

Mode of action. Erythromycin produces its antibacterial activity by inhibiting bacterial protein synthesis through binding to the 50S ribosomal subunit (18). The ability of

TABLE 3. Susceptibility of *Enterobacteriaceae* and other aerobic gram-negative species to azithromycin

Organism (no. of strains)	% of strains inhibited	MIC (μg/ml)		
		Azithromycin	Erythromycin	Cephalexin
Escherichia coli (22)	50	2	32	4.0
	90	4	>64	4.0
Salmonella enteritidis (16)[a]	50	2	64	2.0[b]
	90	4	128	>256
Shigella sonnei (15)[a]	50	1	16	2.0[b]
	90	2	128	128
Shigella flexneri (5)	50	1.0	16.0	2.0[b]
Yersinia enterocolitica (32)	50	0.78	6.25	ND[c]
	90	3.12	>50	
Klebsiella pneumoniae (16)	50	8	64	4.0
	90	16	>64	8.0
Klebsiella oxytoca (11)	50	8	>64	ND
	90	16		
Enterobacter aerogenes (23)	50	4	>64	32
	90	8		>64
Enterobacter cloacae (31)	50	8	>64	>64
	90	16		
Serratia marcescens (18)	50	64	>64	>64
Proteus mirabilis (14)	50	>64	>64	8.0
Proteus vulgaris (12)	50	>64	>64	>64
Citrobacter freundii (19)	50	8	>64	>64
	90	16		
Citrobacter diversus (6)	50	2	64	8.0
Acinetobacter calcoaceticus (13)	50	0.25	2	ND
	90	4.0	8	
Pseudomonas aeruginosa (2)	50	>64	>64	>64

[a] MIC_{90} of tetracycline was 4 μg/ml against *Salmonella enteritidis* and 128 μg/ml against *Shigella sonnei*.
[b] Ampicillin not cephalexin.
[c] ND, Not done.

azithromycin and erythromycin to inhibit natural mRNA-directed cell-free polypeptide synthesis is presented in Fig. 3. Both macrolides were equivalent in activity when ribosomes from the erythromycin-susceptible *S. aureus* strain were used. The concentrations of azithromycin and erythromycin causing 50% inhibition were approximately equal to their MICs against the strains from which the ribosomes were isolated. This correlation has been observed previously with oleandomycin derivatives for inhibition of natural mRNA-directed cell-free protein synthesis with an *E. coli* cell-free system (5). When ribosomes from a constitutive macrolide-resistant strain were used, the degree of inhibition produced by both compounds was substantially reduced even at a 1,000-fold greater concentration (Fig. 3). Azithromycin competed for [^{14}C]erythromycin ribosome-binding sites better than [^{12}C]erythromycin did (Fig. 4), suggesting that it binds to the same receptor as erythromycin.

TABLE 4. In vitro activity of azithromycin against clinical isolates of gram-positive bacteria

Organism (no. of strains)	% of strains inhibited	MIC (μg/ml)	
		Azithromycin	Erythromycin
Staphylococcus aureus[a] (100)	50	0.78	0.20
	90	1.56	0.39
S. aureus, erythromycin resistant (16)	50	>50	>50
Staphylococcus epidermidis[a] (17)	50	0.78	0.20
	90	0.78	0.20
S. epidermidis, erythromycin resistant (12)	50	>50	>50
Streptococcus pyogenes (17)	50	0.1	≤0.025
	90	0.1	≤0.025
Streptococcus pyogenes, erythromycin resistant (7)	50	>50	6.25
	90		>50
Streptococcus pneumoniae (10)	50	≤0.025	≤0.025
	90	0.05	≤0.025
Streptococcus agalactiae (54)	50	0.05	≤0.025
	90	0.10	0.05
Streptococcus faecalis (64)	50	1.56	0.78
	90	>50	>50

[a] The MIC_{50} and MIC_{90} of josamycin against the erythromycin-susceptible *Staphylococcus* strains were both 1.56 μg/ml (both species); against resistant strains, they were 3.12 μg/ml for both species and 12.5 μg/ml and >50 μg/ml for *S. aureus* and *S. epidermidis*, respectively.

DISCUSSION

Recently, Aronoff et al. (1) reported in vitro activities for azithromycin similar to the data reported here. They showed that azithromycin possessed potency against gram-positive organisms similar to that of erythromycin. They also found azithromycin to be fourfold more potent than erythromycin against *H. influenzae* type b and *B. catarrhalis* and twofold more potent against *H. influenzae* non-type b and *H. parainfluenzae*. Thus, azithromycin appears to have an in vitro spectrum and potency suitable for respiratory, otitis media, and skin infections in the outpatient.

Azithromycin was reported to be twice as potent as erythromycin against *N. gonorrhoeae* and *Mycoplasma hominis* and equal to erythromycin against *Ureaplasma urealyticum* and *C. trachomatis* (R. W. Ryan, J. Kwasnik, and R. C. Tilton, 26th ICAAC, abstr. no. 932, 1986). Ridgway (16) also showed azithromycin to be active against *M. hominis* (MIC_{90}, 4 μg/ml, versus MIC_{50} of erythromycin of >32 μg/ml). Walsh et al. (19) reported that azithromycin inhibits 100% inclusion formation by *C. trachomatis* strains

TABLE 5. In vitro activity of azithromycin against anaerobic organisms

Organism (no. of strains)	% of strains inhibited	MIC (μg/ml)			
		Azithromycin	Erythromycin	Amoxicillin	Cefaclor
Bacteroides fragilis (58)	50	3.12	1.56	25[a]	50[b]
	90	6.25	1.56	50	>200
Clostridium perfringens (13)	50	0.78	1.56	≤0.10	0.78
	90	0.78	1.56	≤0.10	0.78
Clostridium difficile (20)	50	3.12	0.39	0.78	25
	90	6.25	1.56	1.56	25
Peptococcus sp. (12)	50	1.56	3.12	≤0.10	0.20
	90	3.12	3.12	0.78	0.78
Peptostreptococcus sp. (3)	67	0.78	0.20	0.39	3.12
Eubacterium limosum (3)	67	25	>50	0.19	3.12
Eubacterium lentum (2)	100	1.56	1.56	1.56	6.25
Other anaerobes[c] (4)	75	≤0.10	≤0.10	≤0.10	0.20

[a] Ampicillin, not amoxicillin.
[b] Cephalexin, not cefaclor.
[c] One strain each of *Propionibacterium acnes*, *Actinomyces naeslundii*, *Actinomyces israelii*, and *Fusobacterium necrophorum*.

at concentrations from 0.26 to 1.02 μg/ml, equivalent to erythromycin. R. C. Brunham of the University of Manitoba (personal communication) also reports azithromycin to have better potency than erythromycin against *N. gonorrhoeae* and *Haemophilus ducreyi*. This activity of azithromycin strongly indicates its use for the treatment of many sexually transmitted bacterial infections.

Czinn et al. (4) have reported an MIC_{90} of azithromycin against *Campylobacter pyloridis* of 0.25 μg/ml; we observed an MIC_{90} of 0.5 μg/ml for a *Campylobacter* sp. (Table 1). The data in Table 4 illustrate the notable in vitro potency advantage of azithromycin over erythromycin against the *Enterobacteriaceae* that cause gastrointestinal diarrheas. Possibly, azithromycin could have broader indications than erythromycin for gastrointestinal infections.

Comparing the published data on erythromycin and two other new macrolides, A-56268 and roxithromycin (RU 28965), with those on azithromycin it becomes apparent that azithromycin has the best potency against gram-negative organisms. Roxithromycin has been reported to be fourfold less active than erythromycin versus *H. influenzae* (11, 12) and twofold less active versus *N. gonorrhoeae* (11); like erythromycin, it is not active against the *Enterobacteriaceae* (11). A-56268 is less active than erythromycin against *H. influenzae* (twofold) and *Enterobacteriaceae*, but equivalent to erythromycin versus *N. gonorrhoeae* (3, 7). Against gram-positive strains, A-56268 appears to be slightly more potent than erythromycin (3, 7), while roxithromycin has about one-half the potency of erythromycin (1, 11). Like azithromycin, A-56268 and roxithromycin show complete cross-resistance with erythromycin-resistant gram-positive cocci (1, 3, 11).

Resistance to erythromycin in gram-positive organisms, whether inducible or constitutive, is due to methylation of an adenine residue in the rRNA in the 50S ribosomal subunit (13). The data in Fig. 3 clearly indicate that azithromycin, like erythromycin, does not interact well with the methylated ribosome, explaining the observed cross-resistance. Erythromycin-resistant *Staphylococcus* strains showed complete cross-resistance to azithromycin, not partial resistance as observed for the 16-membered-ring macrolide josamycin (Table 4). This indicates that azithromycin induces adenine methylation in a manner similar to the 14-membered-ring macrolides (erythromycin and oleandomycin). The better ability of azithromycin than of [^{12}C]erythromycin to compete for [^{14}C]erythromycin-binding sites might indicate a higher affinity of azithromycin for the susceptible ribosome. In an in vivo situation, this would favor an equilibrium of azithromycin going from the tissue to the ribosome of the infecting organism, as observed in the potent activity against *L. pneumophila* grown in lung cell monolayers.

The bactericidal activity of azithromycin needs to be further explored. The data presented in Table 6 and Fig. 2 indicate that azithromycin has better bactericidal activity than erythromycin, although the killing-kinetics experiments show that the killing activity is slow to develop at the MIC. Fernandes et al. (7) also showed that erythromycin and A-56268 are bactericidal for *Streptococcus pyogenes* but not *S. aureus*; roxithromycin is not bactericidal for *S. aureus* (11). Despite the slow bactericidal effect, azithromycin may be bactericidal in vivo because of the high concentration achieved in tissue. The rat lung levels of azithromycin, measured 24 h after a single 10-mg/kg oral dose, well exceed the MIC_{90} of azithromycin against *S. aureus* and *H. influenzae* (9).

TABLE 6. Bactericidal activity of azithromycin and erythromycin compared

Organism (no. of strains)	% of strains inhibited	MIC/MBC (μg/ml)		MBC/MIC (log_2)	
		Azithromycin	Erythromycin	Azithromycin	Erythromycin
Streptococcus pyogenes (15)	50	0.063/0.13	0.016/0.063	1	2
	90	0.13/0.25	0.031/0.13	1	2
Staphylococcus aureus (5)	Average	0.39/6.3	0.1/1.6	4	4
Haemophilus influenzae (10)	50	0.39/0.39	0.78/12.5	0	4
	90	0.39/0.78	3.12/25	1	3
S. aureus (5)[a]	Average	0.10/12.5	1.56/>50	6	6

[a] Tested with dicloxicillin instead of azithromycin and cefaclor instead of erythromycin.

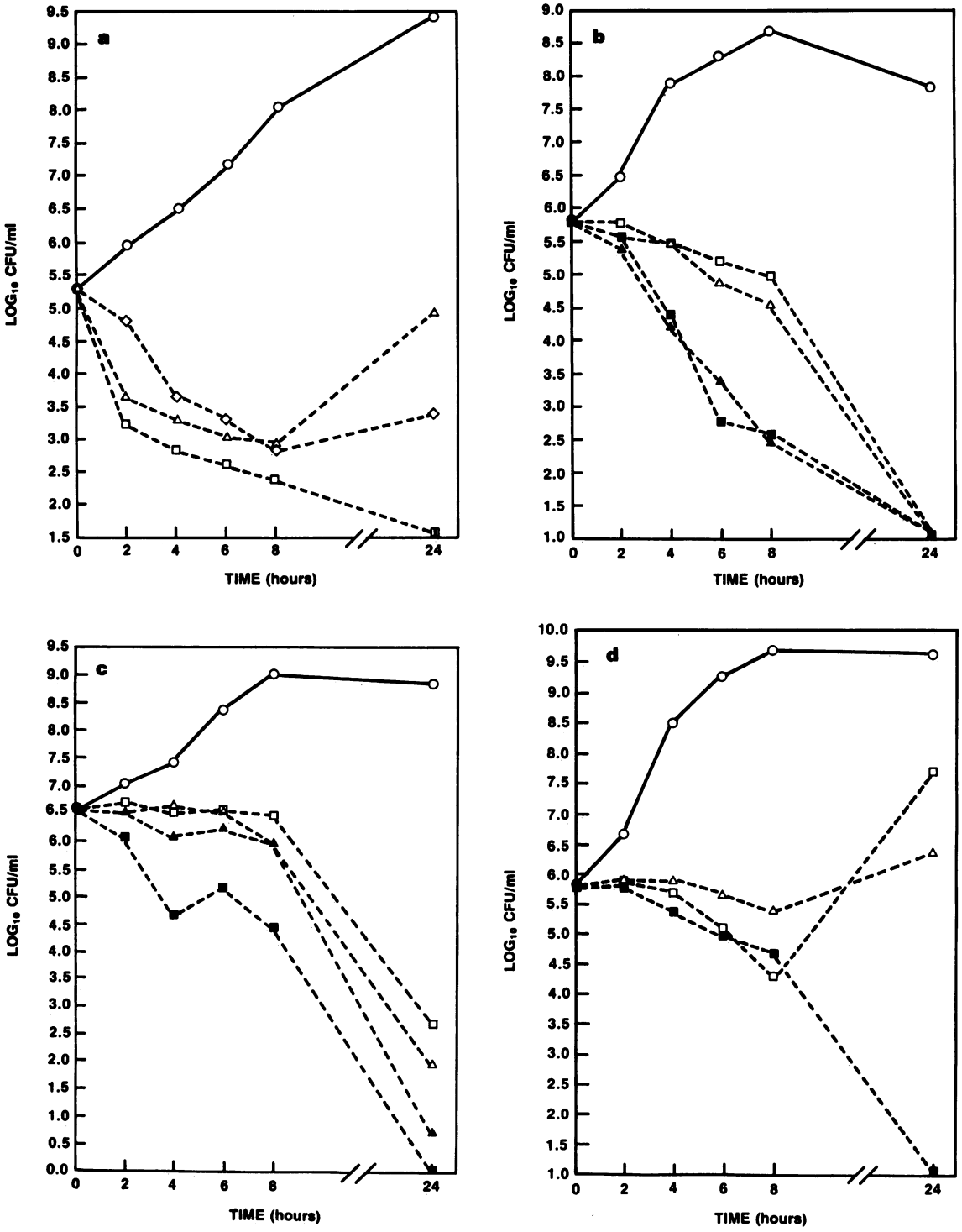

FIG. 2. Bactericidal activity of azithromycin and erythromycin against erythromycin-susceptible strains of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Haemophilus influenzae*, and *Escherichia coli*. (a) *S. aureus* 01A005, all antibiotics at four times their respective MICs. Symbols: ○, control (no antibiotic); □, azithromycin at 3.12 μg/ml; △, erythromycin at 0.4 μg/ml; ◇, ampicillin at 0.8 μg/ml. (b) *Streptococcus pyogenes* 02C133, antibiotics at or four times their MICs. Symbols: ○, control (no antibiotic); □ and ■, azithromycin at 0.125 (□) and 0.5 (■) μg/ml; △ and ▲, erythromycin at 0.0312 (△) and 0.125 (▲) μg/ml. (c) *H. influenzae* 54A042, antibiotics at or four times their MICs. Symbols: ○, control (no antibiotic); □ and ■, azithromycin at 0.78 (□) and 3.12 (■) μg/ml; △ and ▲, erythromycin at 3.12 (△) and 12.5 (▲) μg/ml. (d) *E. coli* 51A511. Symbols: ○, control (no antibiotic); □ and ■, azithromycin at 3.12 (□) and 12.5 (■) μg/ml (MIC and four times the MIC, respectively); △, erythromycin at its MIC of 50 μg/ml.

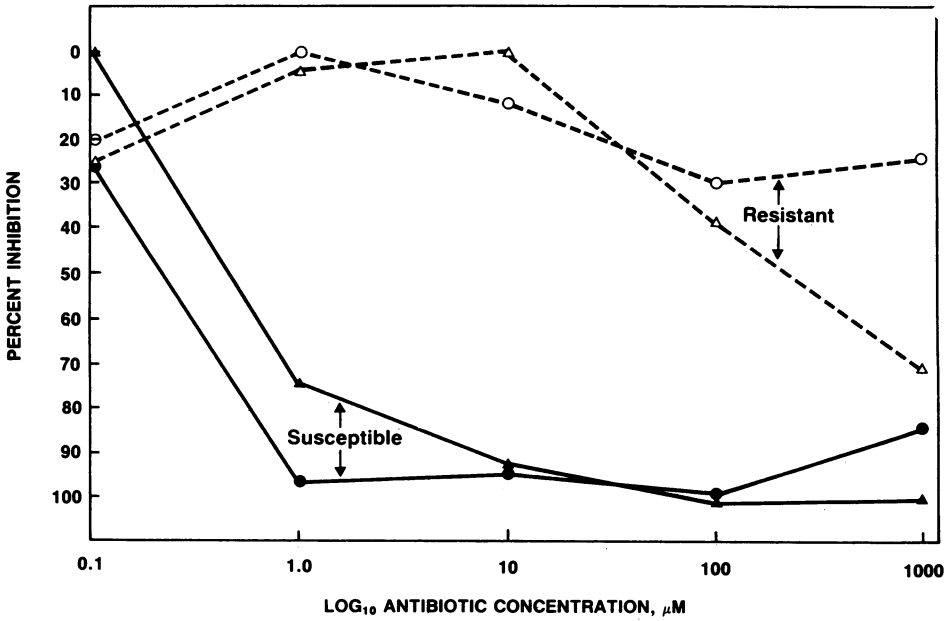

FIG. 3. Inhibition of natural mRNA-directed protein synthesis with *Staphylococcus aureus* resistant and susceptible ribosomes. Symbols: ●, ▲, ribosomes isolated from a susceptible strain (MIC of both antibiotics was 0.27 μM); ○, △, ribosomes isolated from a constitutively resistant strain (MIC, 272 and 267 μM for erythromycin and azithromycin, respectively); ●, ○, erythromycin; ▲, △, azithromycin.

In summary, the in vitro and especially the pharmacokinetic and in vivo properties of azithromycin (9) compared with those of erythromycin suggest that additional laboratory and clinical research on this interesting macrolide is warranted to test its potential for the oral treatment of respiratory, cutaneous, and sexually transmitted infections, closed-space infections, and gastrointestinal diarrheas and see whether its indications can be extended beyond those of erythromycin.

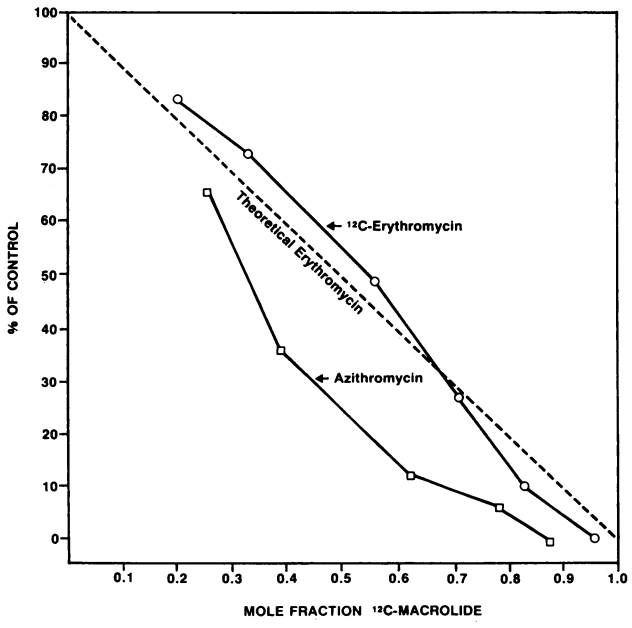

FIG. 4. Azithromycin inhibition of [^{14}C]erythromycin binding to ribosomes from an erythromycin-susceptible *Staphylococcus aureus* strain. Dashed line, Calculated line for [^{12}C]erythromycin competing with [^{14}C]erythromycin.

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