Efficacies of ABT-773, a New Ketolide, against Experimental Bacterial Infections

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ABT-773 is a novel ketolide effective against antibacterial-resistant respiratory tract pathogens. The pharmacokinetic profile of ABT-773 was studied in rats and consisted of a mean peak concentration in plasma of 1.07 μg/ml and an area under the concentration-time curve (AUC) of 12.03 μg · h/ml when the compound was delivered at a dose of 25 mg/kg of body weight. It concentrated in rat lung tissue, with a lung tissue-to-plasma ratio of 29 based on the AUC. In acute systemic infections in mice, ABT-773 showed efficacy against macrolide-susceptible strains of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *S. pyogenes*, and *Listeria monocytogenes*. Additionally, ABT-773 improved the survival of mice infected with resistant *S. pneumoniae* containing either the *ermB* gene, the *mefE* gene, or altered penicillin binding protein genes. In a rat lung model of infection, ABT-773 demonstrated 50% effective doses lower than those of comparator macrolides when evaluated against the following strains of *S. pneumoniae*: a macrolide-lincosamide-streptogramin B-susceptible strain, an *ermB* strain, and an *mefE* strain. ABT-773 was also effective against *Haemophilus influenzae* lung infections in rats. Thus, ABT-773 may prove to be a useful new antibacterial agent for the treatment of respiratory tract infections.

Multidrug-resistant *Streptococcus pneumoniae* is a major public health concern (7, 11, 14, 15, 17, 30, 31, 34). Significant morbidity and mortality occur from community-acquired pneumonia, meningitis, otitis media, and sinusitis. *S. pneumoniae* isolates are macrolide resistant due to the presence of either *ermB* or *mefE* determinants (20, 21, 26). The streptococcal *ermB* gene encodes an enzyme which methylates the ribosome, thus blocking macrolide binding. This leads to high-level resistance to macrolides-lincosamides-streptogramin B (MLS$_B$ resistance) (erythromycin MIC ≥ 64 μg/ml). The *mefE* gene encodes an active macrolide efflux pump which is associated with low-level resistance to 14- and 15-membered-ring macrolides (erythromycin MIC range, 1 to 32 μg/ml) (35). Macrolide resistance rates in *S. pneumoniae* have been reported to vary in different epidemiological settings (21, 30).

Resistance is not limited to streptococci. Thirty to forty percent of nontypeable *Haemophilus influenzae* isolates produce β-lactamase and are resistant to penicillins (16, 33). However, specific resistance mechanisms for macrolide resistance in *H. influenzae*, other than factors related to intrinsic activity, are rare.

As bacterial resistance increases, there is need for new effective agents. Ketolides may provide physicians an additional therapeutic option (1, 5, 6, 8, 9, 10, 27). ABT-773, a novel ketolide synthesized at Abbott Laboratories, was designed to have activity against major resistant respiratory pathogens. The

key chemical modifications to the erythromycin A core are a quinolylally moiety attached to the 6-O position, a keto group at the 3 position, and a cyclic carbamate group residing at the 11,12 position (Fig. 1). These structural changes have led to increased antimicrobial activity and improvements in the pharmacokinetic profile (25; Z. Ma, R. Clark, S. Wang, A. Nilius, R. Flamm, and Y. Or, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2133, 1999).

The purpose of this paper is to report the therapeutic profile of ABT-773 in animal models. The value of these models is in providing insight through an overall assessment of factors influencing drug efficacy, such as potency, pharmacokinetics, and tissue penetration.

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MATERIALS AND METHODS

Antimicrobial agents. ABT-773 and clarithromycin were synthesized at Abbott Laboratories, Abbott Park, Ill. Azithromycin was obtained from Pfizer Labs (New York, N.Y.), and penicillin G was obtained from Wyeth Laboratories Inc. (Philadelphia, Pa.).

Bacterial strains. All bacterial strains were clinical isolates maintained in the culture collection of Abbott Laboratories or were obtained from the American Type Culture Collection (Manassas, Va.).

In vitro susceptibility test. MICs for *Staphylococcus aureus*, *S. pneumoniae*, *Streptococcus pyogenes*, and *H. influenzae* were determined by broth microdilution methods described by the National Committee for Clinical Laboratory Standards (NCCLS). MICs for *Listeria monocytogenes* were determined by the agar dilution method on Mueller-Hinton agar supplemented with 5% sheep blood after incubation for 16 to 18 h at 35°C in ambient air (22).

In vivo tests. The efficacy of ABT-773 was compared to that of penicillin and/or clarithromycin in murine models of acute bacterial infections as described previously (3, 12). In brief, CF1 female mice (Charles River, Wilmington, Mass.)

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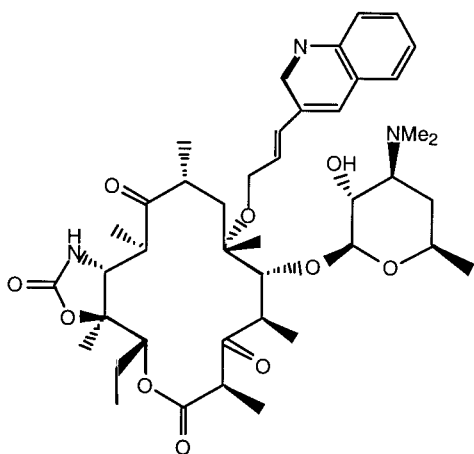§ Present address: Enanta Pharmaceuticals, Cambridge, MA 02139.

FIG. 1. Structure of ABT-773 (molecular weight, 765,953).

weighing 20 to 25 g were inoculated intraperitoneally with overnight culture dilutions in normal saline of *S. pyogenes* C203, *L. monocytogenes* PIU 2526, *S. pneumoniae* 6303, and *S. pneumoniae* 6396. The remaining organisms with low virulence, *S. aureus* NTCC 10649, *S. pneumoniae* 6502, *S. pneumoniae* 5649, *S. pneumoniae* 5654, *S. pneumoniae* 5669, and *S. pneumoniae* 5979, were enhanced by diluting in 5% hog gastric mucin (American Laboratories, Omaha, Nebr.). All cultures were adjusted to yield approximately 100 times the 50% lethal dose (LD$_{50}$). Concurrently with each trial, the LD$_{50}$ was confirmed by inoculation of untreated mice with log$_{10}$ dilutions of bacterial inoculum over a 5-log$_{10}$ dilution range (10 mice per log$_{10}$ dilution). ABT-773 was formulated in 2% ethanol with 1 mol equivalent of HCl (vol/vol) in 5% sterile dextrose water and was dosed orally. Clarithromycin was formulated in 2% ethanol (vol/vol) in phosphate-buffered saline (PBS), pH 6.8, and was dosed orally. Penicillin G was formulated in normal saline for injection and was administered by the subcutaneous route. For trials with *S. aureus*, *S. pyogenes*, and *S. pneumoniae*, mice were treated 1 and 5 h postchallenge. For the *L. monocytogenes* infection, therapy was delayed for 24 h to allow establishment of an intracellular infection, and compounds were administered 24 and 28 h postinoculation. Mortality and/or morbidity was recorded for 7 days, and the mean effective dose sufficient to protect 50% of the mice (ED$_{50}$) was calculated from the cumulative mortality by trimmed-logit analysis (19, 32). There were 10 mice per dosage group. To evaluate drug efficacy in an organ-specific model of infection, ABT-773 was tested with clarithromycin and azithromycin in pulmonary infection models in rats (4, 18). Sprague-Dawley CD male rats (Charles River) weighing 190 to 230 g were inoculated intratracheally with 0.5 ml containing 10^5 to 10^8 CFU of *S. pneumoniae* or *H. influenzae* in 5% hog gastric mucin. A stainless steel feeding needle (20-gauge by 3 in., with a 2.25-mm-diameter ball) with the last 1 cm bent to a 45° angle was used to deliver the inoculum. Groups of 10 infected rats were treated with vehicle and served as the infected controls. Clarithromycin and ABT-773 were formulated as previously mentioned, while azithromycin was formulated in 2% ethanol (vol/vol) in PBS, pH 6.8. Three consecutive days of therapy with either ABT-773, azithromycin, or clarithromycin was initiated 18 h postinoculation with *S. pneumoniae* and 5 h postinoculation with *H. influenzae*. Oral treatments with ABT-773 or azithromycin were administered once daily (q.d.), while clarithromycin was administered twice daily (12 h apart). Delivering the total daily dose of clarithromycin 12 h apart gives a more accurate representation of its efficacy and has potential to increase the time at which the concentrations remain above the MIC. Twice-daily dosing or dosing with extended-release formulation of clarithromycin and once-a-day dosing with azithromycin are methods representative of how these drugs are administered in humans. Preliminary evaluations of ABT-773 suggested only a slight difference in efficacy between twice daily and once-a-day dosing. Drug efficacy was determined by measuring the reduction of bacterial burden compared to that observed in vehicle-treated controls. Lungs were removed from the animals 12 h after the last clarithromycin treatment or 24 h after the last ABT-773 or azithromycin treatment on day 4 for *S. pneumoniae* and day 3 for *H. influenzae* infections. Lungs were aseptically removed and homogenized in 5 ml of sterile PBS with a sterile tissue homogenizer (Tekmar, Cincinnati, Ohio). Bacterial burden was assessed from log dilution plating (1/50 to 1/500,000) of the homogenates on chocolate agar plates for *H. influenzae* and Columbia agar-nalidixic acid plates for *S. pneumoniae*. Agar plates

were incubated at 37°C in 5% CO$_2$ for 24 h to allow the visualization and counting of colonies. The minimum number of bacteria detectable by this method is 50 CFU/lung pair. Calculation of asymptotic standard errors and analysis of variance were conducted for the dosage groups, and 95% confidence intervals were generated for ED$_{50}$s for producing a 2- or 3-log$_{10}$ reduction in bacteria compared to the mean burden observed in the vehicle-treated control (2, 28). The analysis of bacterial reduction is an acceptable interpretation of results obtained from an organ burden trial. An analysis based only on bacterial clearance or cures focuses on the maximum effective doses while excluding the rest of the dosage-response curve. Determining the drug dose required to show a 2- or 3-log reduction allows more influence by individual animals which show multiple-log reductions but are not cured.

Pharmacokinetics. Single- and multiple-oral-dose pharmacokinetic studies were conducted with 190- to 230-g Sprague-Dawley CD male rats (Charles River). ABT-773 was solution formulated in 2% ethanol with 1 mol equivalent of HCl (vol/vol) in 5% sterile dextrose water. Clarithromycin and azithromycin were formulated in 2% ethanol (vol/vol) in PBS, pH 6.8. The dosages of each compound were selected as those which generally produce a 2-log reduction of *H. influenzae* using the pulmonary infection model in rats. In the multidose trial, ABT-773 and azithromycin were administered every 24 h, for a total of three dosings at 25 and 50 mg/kg of body weight, respectively. Clarithromycin was administered at 12-h intervals, with a total of five dosings at 50 mg/kg. Plasma and lung samples were collected at 0.5, 1, 2, 3, 6, 9, 12, and 24 h after the last dose. Three rats were used for each time point. The entire lung tissues were removed, weighed, rinsed free of blood with 0.9% NaCl, diluted 1:2 (wt/vol) in distilled water, and homogenized thoroughly. A 0.5-ml aliquot is extracted using the same methods used for plasma (see below). A hemoglobin count was determined to account for blood contribution to drug tissue levels. Blood samples were chilled immediately and centrifuged under refrigeration, and the plasma, along with lung tissue homogenates, was frozen within 1 h of collection. Drug concentrations in lung homogenate and plasma were determined by high-pressure liquid chromatography. Samples of 0.2 to 0.5 ml of plasma plus 50 μl of internal standard (proprietary ketolide) plus 0.5 ml of 0.5 M Na$_2$CO$_3$ were extracted with 6.0 ml of ethyl acetate-hexane (1:1, vol/vol). The organic layer was evaporated to dryness with nitrogen at room temperature while protected from light. Samples were reconstituted with acetonitrile–0.05 M phosphate buffer, pH 6.0 (1:2, vol/vol). Chromatographic conditions consisted of a YMC basic column (250 mm by 4.6 mm by 5 μm) with an acetonitrile:methanol-buffer-mobile phase (45:5:60, by volume) at a flow rate of 1.0 ml/min. The mobile phase buffer (pH 6.0) contained 0.01 M tetramethylammonium hydroxide in 0.05 M KH$_2$PO$_4$. Detection of analytes was accomplished by fluorescence at an excitation wavelength of 324 nm and an emission wavelength of 364 nm. The analytical method was linear over the concentration range 0.005 to 5.18 μg/ml. The precision expressed, as relative standard deviation, was <2.9% for triplicate analysis. The efficiency of extraction averaged 89.5%.

RESULTS

Efficacies of ABT-773 against infections caused by macrolide-susceptible respiratory and intracellular pathogens in mice. In these studies, a mortality rate of 100% for each infection was produced in all groups of untreated mice. The ED$_{50}$s of ABT-773 were consistently lower than those of clarithromycin against infections caused by macrolide-susceptible respiratory or intracellular pathogens (Table 1). Against *S. aureus*, the oral ED$_{50}$ of ABT-773 was 9.4 mg/kg, compared to 30.2 mg/kg for clarithromycin. The ED$_{50}$s of ABT-773 were approximately threefold lower than those of clarithromycin against *S. pneumoniae* (10.0 and 37.3 mg/kg, respectively). The ED$_{50}$s generated for ABT-773 and clarithromycin against *S. pyogenes* were not significantly different, and the 95% confidence limits overlapped (1.9 and 3.7 mg/kg, respectively). The ED$_{50}$ of ABT-773 was 100.1 mg/kg for intracellular infections with *L. monocytogenes*, while no efficacy was observed at the highest clarithromycin dose (200 mg/kg).

Efficacies of ABT-773 against systemic infections caused by resistant *S. pneumoniae* in mice. The relative efficacies of ABT-773 and the reference standards, clarithromycin and penicillin,

TABLE 1. Oral efficacy of ABT-773 against important respiratory and intracellular bacterial infections in mice[b]

Bacterium and compound	MIC (μg/ml)	ED_{50}[a] (95% confidence limits)
S. aureus 10649[c]		
ABT-773	0.03	9.4 (6.0–14.8)
Clarithromycin	0.25	30.2 (20.9–43.8)
S. pneumoniae 6303[d]		
ABT-773	0.001	10.0 (5.5–18.2)
Clarithromycin	0.008	37.3 (22.8–60.8)
S. pyogenes C203[e]		
ABT-773	0.002	1.9 (1.1–3.3)
Clarithromycin	0.03	3.7 (2.3–6.1)
L. monocytogenes 2526[f]		
ABT-773	0.03	100.1 (63.2–158.4)
Clarithromycin	0.12	>200 (NC[g])

[a] Dosage (in milligrams per kilogram per day) required to protect 50% of the mice against inoculation with 100 times the LD_{50}.
[b] Groups of untreated mice showed 100% mortality.
[c] Challenge inoculum of log_{10} 5.1.
[d] Challenge inoculum of log_{10} 2.3.
[e] Challenge inoculum of log_{10} 5.4.
[f] Challenge inoculum of log_{10} 6.6.
[g] NC, not calculated.

TABLE 2. Oral efficacy of ABT-773 against bacterial infections caused by resistant *S. pneumoniae*[b] in mice

Strain (characteristics) and compound	MIC (μg/ml)	ED_{50}[a] (95% confidence range)
6502[c] (MLS^s Pen^r)		
ABT-773	0.001	12.4 (7.3–21.0)
Clarithromycin	0.004	16.5 (9.0–30.3)
Penicillin	2	167.4 (99.0–283.0)
5649[d] (*mefE*, Pen^i)		
ABT-773	0.06	50.1 (31.6–79.2)
Clarithromycin	4	>100 (NC[i])
Penicillin	0.5	<37.5 (NC)
5654[e] (*mefE*, Pen^r)		
ABT-773	0.06	52.8 (27.2–102.5)
Clarithromycin	4	>100 (NC)
Penicillin	2	108.9 (71.5–165.8)
6396[f] (*ermB*, Pen^s)		
ABT-773	0.015	50.1 (31.6–79.2)
Clarithromycin	>128	>200 (NC)
Penicillin	0.06	22.2 (14.1–35.0)
5669[g] (*ermB*, Pen^i)		
ABT-773	0.015	14.7 (6.0–36.0)
Clarithromycin	>128	123.8 (70.2–218.4)
Penicillin	1	24.1 (12.5–46.4)
5979[h] (*ermB*, Pen^r)		
ABT-773	0.015	10.3 (7.1–15.0)
Clarithromycin	>128	>200 (NC)
Penicillin	4	>800 (NC)

[a] Dosage (in milligrams per kilogram per day) required to protect 50% of the mice against inoculation with 100 times the LD_{50}.
[b] Groups of untreated mice showed 100% mortality.
[c] Challenge inoculum of log_{10} 7.1.
[d] Challenge inoculum of log_{10} 6.4.
[e] Challenge inoculum of log_{10} 6.2.
[f] Challenge inoculum of log_{10} 2.4.
[g] Challenge inoculum of log_{10} 5.6.
[h] Challenge inoculum of log_{10} 5.4.
[i] NC, not calculated.

against acute infections in mice caused by resistant *S. pneumoniae* are reported in Table 2. Against *S. pneumoniae* strain 6502, which is MLS_B susceptible and penicillin resistant, the ED_{50}s of ABT-773, clarithromycin, and penicillin were 12.4, 16.6, and 167.4 mg/kg, respectively. Against *S. pneumoniae* strain 5649, which is resistant to macrolides via the *mefE* gene and is penicillin intermediate, the ED_{50}s of ABT-773, clarithromycin, and penicillin were 50.1, >100, and <37.5 mg/kg, respectively. Against *S. pneumoniae* strain 5654, which also has an *mefE* gene and is penicillin resistant, the ED_{50}s of ABT-773, clarithromycin, and penicillin were 52.8, >100, and 108.9 mg/kg, respectively. Against *S. pneumoniae* strain 6396, which shows constitutive macrolide resistance due to the presence of an *ermB* gene and is penicillin susceptible, the ED_{50}s generated for ABT-773, clarithromycin, and penicillin were 50.1, >200, and 22.2 mg/kg. Against *S. pneumoniae* strain 5669, which also has an *ermB* gene and is penicillin intermediate, the ED_{50}s of ABT-773, clarithromycin, and penicillin were 14.7, 123.8, and 24.1 mg/kg, respectively. Against *S. pneumoniae* strain 5979, which has an *ermB* gene and is penicillin resistant, ABT-773 demonstrated 50% protection of mice at 10.3 mg/kg while clarithromycin and penicillin showed no efficacy at the highest dosages given (200 and 800 mg/kg, respectively).

Efficacy of ABT-773 against *S. pneumoniae*-associated lung infections in rats. The efficacy of ABT-773 was consistently better than those of the comparative macrolides, azithromycin, and clarithromycin against pulmonary infection in rats caused by susceptible or resistant (*mefE* or *ermB*) strains of *S. pneumoniae* (Table 3). After three consecutive days of oral dosing at 2.5 mg/kg/day, ABT-773 produced a mean 4.68-log_{10} reduction in bacterial lung burden compared to the vehicle-treated control group ($P < 0.001$) when an MLS_B-susceptible stain (ATCC 6303) was tested. The ED_{50}s that produced a 3-log_{10} reduction in viable bacterial count compared to the vehicle-

treated controls for ABT-773, azithromycin, and clarithromycin were 1.2, 6.0, and 6.2 mg/kg/day, respectively, for this strain. Against the macrolide-resistant *S. pneumoniae* strain 5649, which has an efflux pump encoded by the *mefE* gene, ABT-773 produced a mean 4.45-log_{10} reduction in lung burden compared to the vehicle-treated control group when administered at 20 mg/kg/day ($P < 0.001$). The 3-log_{10} reduction ED_{50} of ABT-773 was 15.1 mg/kg/day, while the ED_{50}s of azithromycin and clarithromycin were 78.7 and 69.7 mg/kg/day, respectively. *S. pneumoniae* strain 6396 has an *ermB* gene that causes constitutive MLS_B resistance. The MICs of azithromycin and clarithromycin were >128 μg/ml. There was no significant reduction in bacterial lung burden in rats inoculated with *S. pneumoniae* strain 6396 after treatment with either azithromycin or clarithromycin at 100 mg/kg/day. ABT-773 (MIC, 0.015 μg/ml) produced a significant reduction in lung burden when administered at 10 mg/kg/day ($P < 0.001$). The 3-log_{10} reduction ED_{50} of ABT-773 was 2.3 mg/kg/day.

Efficacy of ABT-773 against *H. influenzae*-associated lung infections in rats. The efficacy of ABT-773 was consistently improved over those of the comparative macrolides, azithromycin, and clarithromycin against pulmonary infection in rats

TABLE 3. Efficacies of ABT-773 and comparative agents in experimental *S. pneumoniae*-associated lung infections in rats

S. pneumoniae strain (characteristic) and compound	Daily dose[e] (mg/kg)	MIC (μg/ml)	Log_{10} CFU/lung (mean $\pm$ SE)	P[g]	ED$_{50}$[h] (95% confidence limits) 2-log_{10} reduction	3-log_{10} reduction
6303 (MLS$_B$^{s})[a]						
Vehicle control[d]	NA[i]	NA	8.13 $\pm$ 0.08		NA	NA
ABT-773	0.6	0.001	5.89 $\pm$ 0.67	0.029	<0.6 (NC)[j]	1.2 (1.2–1.3)
	2.5		3.45 $\pm$ 0.25	<0.001		
	10		3.27 $\pm$ 0.19	<0.001		
Azithromycin	3.1	0.03	6.38 $\pm$ 0.52	0.027	3.7 (2.3–5.9)	6.0 (3.8–9.6)
	12.5		2.33 $\pm$ 0.82	0.002		
	50		1.14 $\pm$ 0.47	<0.001		
Clarithromycin[f]	3.1	0.008	6.38 $\pm$ 0.85	0.109	3.7 (2.3–6.2)	6.2 (3.8–10.3)
	12.5		2.60 $\pm$ 0.82	0.002		
	50		2.33 $\pm$ 0.15	<0.001		
5649 (*mefE*)[b]						
Vehicle control	NA	NA	5.37 $\pm$ 0.47		NA	NA
ABT-773	1.25	0.06	4.99 $\pm$ 0.70	0.65	11.8 (6.6–21.1)	15.1 (8.4–27.1)
	5		5.39 $\pm$ 0.97	0.98		
	20		0.92 $\pm$ 0.57	<0.001		
Azithromycin	6.3	8	6.20 $\pm$ 0.70	0.26	24.9 (20.7–30.1)	78.7 (62.2–99.5)
	25		3.37 $\pm$ 0.46	0.019		
	100		1.98 $\pm$ 0.53	<0.001		
Clarithromycin	6.3	4	4.66 $\pm$ 0.22	0.37	41.9 (39.3–44.7)	69.7 (65.3–74.3)
	25		3.98 $\pm$ 0.35	0.083		
	100		1.28 $\pm$ 0.55	<0.001		
6396 (*ermB*)[c]						
Vehicle control	NA	NA	6.48 $\pm$ 0.31		NA	NA
ABT-773	0.6	0.015	4.75 $\pm$ 0.39	0.006	1.0 (0.6–1.6)	2.3 (1.4–3.7)
	2.5		3.29 $\pm$ 0.07	<0.001		
	10		2.81 $\pm$ 0.28	<0.001		
Azithromycin	10	>128	6.75 $\pm$ 0.59	0.66	>100 (NC)	>100 (NC)
	30		5.55 $\pm$ 0.59	0.14		
	100		7.39 $\pm$ 0.63	0.17		
Clarithromycin	10	>128	5.78 $\pm$ 0.64	0.28	>100 (NC)	>100 (NC)
	30		6.61 $\pm$ 0.29	0.79		
	100		7.76 $\pm$ 0.13	0.003		

[a] Intratracheal inoculum of log_{10} 5.8.
[b] Intratracheal inoculum of log_{10} 7.2.
[c] Intratracheal inoculum of log_{10} 5.9.
[d] Vehicle-treated infected control rats were dosed orally for 3 days.
[e] Rats were orally dosed daily for 3 days, starting 18 h postinfection.
[f] Clarithromycin total daily dose given half strength 12 h apart.
[g] Statistical difference for comparison with result for untreated control, determined by the *t* test or Mann-Whitney U test.
[h] The ED$_{50}$ (in milligrams per kilogram per day) to produce a 2- or 3-log_{10} reduction in bacterial count compared to the vehicle-treated controls using linear regression of the group means.
[i] NA, not applicable.
[j] NC, not calculated.

caused by *H. influenzae* (Table 4). Against *H. influenzae* strain 1435, ABT-773 produced a mean 3.32-log_{10} reduction in lung burden compared to the vehicle-treated control group when administered at 60 mg/kg/day for 3 consecutive days ($P < 0.001$). The 2-log_{10} reduction in ED$_{50}$ of ABT-773 was 19.8 mg/kg/day, while the ED$_{50}$s of azithromycin and clarithromycin were 55.4 and 109.8 mg/kg/day, respectively. The antibacterial effect of ABT-773 was demonstrated against *H. influenzae* strain 3643 as a 4.00-log_{10} reduction in lung burden compared to that observed with the vehicle-treated control group when administered at 60 mg/kg/day ($P = 0.002$). The 2-log_{10} reductions in ED$_{50}$s of ABT-773, azithromycin, and clarithromycin were 29.0, 44.2, and 67.8 mg/kg/day, respectively.

Pharmacokinetics. The concentrations of ABT-773 in plasma and lung were determined after single and multiple (once daily [q.d.] for 3 days) oral dosing of 25 mg/kg in rats

(Fig. 2). The single-dose maximum concentration of drug (C_{max}) and area under the concentration-time curve (AUC) in plasma were 1.07 μg/ml and 12.03 μg · h/ml, respectively, while the C_{max} and AUC in lung were 32.11 μg/ml and 350.5 μg · h/ml, respectively (Table 5). After multiple dosing, the C_{max} and AUC in plasma were 0.94 μg/ml and 12.13 μg · h/ml, respectively, while the C_{max} and AUC in lung were 27.10 μg/ml and 351.3 μg · h/ml, respectively. The pharmacokinetics were, therefore, similar between single and multiple dosing. ABT-773 also accumulated to higher concentrations in lung tissue relative to the concentrations in plasma with both single and multiple doses. After multiple dosing (q.d. for 3 days) of azithromycin at 50 mg/kg, the C_{max} and AUC in plasma were 0.97 μg/ml and 14.0 μg · h/ml, respectively, while the C_{max} and AUC in lung were 37.5 μg/ml and 765.9 μg · h/ml, respectively

TABLE 4. Efficacies of ABT-773 and comparative agents in experimental *H. influenzae*-associated lung infections in rats

H. influenzae strain and compound	Daily dose[d] (mg/kg)	MIC (μg/ml)	Log$_{10}$ CFU/lung (mean ± SEM)	P[f]	ED$_{50}$[g] (95% confidence limits) 2-log$_{10}$ reduction	ED$_{50}$[g] (95% confidence limits) 3-log$_{10}$ reduction
1435[a]						
Vehicle control[c]	NA[h]	NA	5.23 ± 0.40		NA	NA
ABT-773	5	0.5	6.38 ± 0.66	0.14	19.8 (15.8–24.8)	50.0 (35.6–70.3)
	20		3.19 ± 0.36	0.006		
	60		1.91 ± 0.51	<0.001		
Azithromycin	5	1	6.17 ± 0.61	0.21	55.4 (33.0–92.8)	70.3 (41.9–117.8)
	25		5.27 ± 1.08	0.97		
	80		1.58 ± 0.65	<0.001		
Clarithromycin[e]	10	2	4.94 ± 0.35	0.65	109.8 (87.8–137.4)	>120 (NC[i])
	40		6.15 ± 0.64	0.23		
	120		2.81 ± 0.16	<0.001		
3643[b]						
Vehicle control	NA	NA	4.50 ± 0.38		NA	NA
ABT-773	5	2	4.86 ± 0.19	0.20	29.0 (28.7–29.2)	44.5 (44.1–44.8)
	20		3.08 ± 0.33	0.02		
	60		0.50 ± 0.50	0.002		
Azithromycin	5	1	4.57 ± 0.30	0.55	44.2 (44.0–44.5)	67.3 (67.0–67.7)
	25		3.33 ± 0.35	0.06		
	80		0.95 ± 0.59	0.002		
Clarithromycin	10	4	4.44 ± 0.30	0.89	67.8 (66.1–69.6)	101.4 (98.8–104.0)
	40		3.33 ± 0.43	0.06		
	120		0.94 ± 0.58	0.002		

[a] Intratracheal inoculum of log$_{10}$ 7.8.
[b] Intratracheal inoculum of log$_{10}$ 8.6.
[c] Vehicle-treated infected control rats were dosed orally for 3 days.
[d] Rats were orally dosed daily for 3 days, starting 18 h postinfection.
[e] Clarithromycin total daily dose given half strength 12 h apart.
[f] Statistical difference for comparison with result for untreated control, determined by the *t* test or Mann-Whitney U test.
[g] The ED$_{50}$ (in milligrams per kilogram per day) to produce a 2- or 3-log$_{10}$ reduction in bacterial count compared to the vehicle-treated controls using linear regression of the group means.
[h] NA, not applicable.
[i] NC, not calculated.

(Table 5; Fig. 3). When clarithromycin was delivered twice a day with five dosages delivered over 48 h, the C_{max} and AUC in plasma were 8.4 μg/ml and 68.1 μg · h/ml, respectively, while the C_{max} and AUC in lung were 51.1 μg/ml and 554.9 μg · h/ml, respectively (Table 5; Fig. 4).

DISCUSSION

To gain a better understanding of the therapeutic potential of ABT-773, evaluations have been undertaken in rodent models of infection. The lethal systemic murine model gives an initial insight into a compound's properties of oral absorption, adequacy of serum concentration, and inhibition of bacterial growth. Mice were inoculated with a bacterial challenge designed to overwhelm the host's capacity to clear the infection without therapy. The net antibacterial effect as measured by increased survival is therefore presumed to be due to the effects of the antimicrobial agent.

The analysis of bacterial reduction is an acceptable interpretation of results obtained from an organ burden trial. An analysis based only on bacterial clearance or cures focuses on the maximum effective doses while excluding the rest of the dosage-response curve. Determining the drug dose required to show 99.0 or 99.9% reduction allows more influence by individual animals which show multiple-log reductions in bacterial burden but are not cured. In an effort to evaluate efficacies of ABT-773 against respiratory pathogens, the rat pulmonary in-
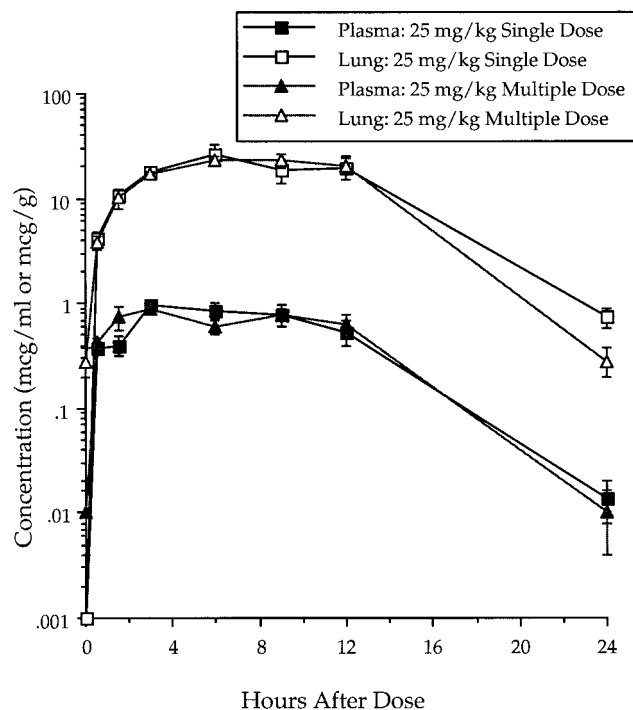

FIG. 2. ABT-773 concentrations in plasma and lung after single and multiple 25-mg/kg oral dosing in rats. Error bars, standard errors.

TABLE 5. Drug concentrations in plasma and lung after single and multiple oral dosing in rats

Compound (dose[a]) and compartment	Single dose					Multiple dose[b]				
	C_{max}[c]	C_{24}[c]	T_{max}[d] (h)	$t_{1/2}$ (h)	AUC_{24}[e]	C_{max}[c]	C_{24}[c]	T_{max} (h)	$t_{1/2}$ (h)	AUC_{24}[e]
ABT-773 (25)										
Plasma	11.07	0.014	6.0	2.8	12.0	0.94	0.010	8.0	2.3	12.1
Lung	32.11	0.746	9.0	3.3	350.6	27.10	0.284	9.0	2.6	351.3
T/P[f]					29.2					29.0
Azithromycin (50)										
Plasma	1.08	0.096	3.0	6.5	8.6	0.97	0.233	3.0	11.6	14.0
Lung	21.41	10.30	9.0	21.4	644.8	37.5	21.17	5.0	7.3	765.9
T/P					75.4					54.7
Clarithromycin (50)										
Plasma	8.20	0.165	3.0	3.15	44.8	8.4	0.168	3.0	8.9	68.1
Lung	55.27	1.105	5.0	3.53	413.8	51.13	1.023	3.3	12.5	554.9
T/P					9.2					8.1

[a] Dose in milligrams per kilogram.
[b] Administered q.d. for 3 days or twice daily for 5 days.
[c] Measured in micrograms per milliliter (plasma) or micrograms per gram (lung).
[d] Time to C_{max}.
[e] Measured in micrograms · hours per milliliter (plasma) or micrograms · hours per gram (lung).
[f] T/P, ratio of tissue AUC_{24} to plasma AUC_{24}.

fection model was used. In experimental rat lung infections caused by *S. pneumoniae* and *H. influenzae*, both a ≥ 2- and a ≥ 3-log$_{10}$ reduction in lung bacterial burden were evaluated. Both ≥ 2- and ≥ 3-log$_{10}$ reductions in bacterial burden have traditionally been considered to be significant reductions in experimental studies (2, 4).

ABT-773 showed improved efficacy over clarithromycin against macrolide-susceptible strains of *S. aureus*, *S. pneumoniae*, and *L. monocytogenes* and equivalent efficacy compared to clarithromycin against *S. pyogenes* in the lethal systemic mouse model. The range in ED$_{50}$s of ABT-773 against

FIG. 3. Azithromycin concentrations in plasma and lung after single and multiple 50-mg/kg oral dosing in rats. Error bars, standard errors.

susceptible respiratory pathogens was 1.9 to 10 mg/kg. ABT-773 was also effective in increasing the survival of mice inoculated with the intracellular bacterium, *L. monocytogenes* (Table 1). The improved efficacy of ABT-773 in comparison to clarithromycin against these gram-positive species correlates with the improved antibacterial activity of the compound in vitro (Table 6) (24).

In addition to its efficacy for treatment of susceptible *S. pneumoniae* in a systemic infection model, ABT-773 also demonstrated a fivefold improvement in ED$_{50}$ compared to other macrolides when evaluated against the susceptible strain of *S. pneumoniae* in the lung infection model in rats. ABT-773 also effectively reduced the bacterial lung burden in rats inoculated with *H. influenzae*. The in vivo potency of ABT-773 was greater than that of azithromycin against *H. influenzae* strain 3643; however, statistical differences were not demonstrated against the *H. influenzae* strain 1435 after comparing ED$_{50}$s of 3-log$_{10}$ reductions (Table 4).

Establishing an experimental *H. influenzae* infection in the rat lung is difficult as the natural host defenses of the rat are capable of clearing the infection. As shown in this study, the initial inoculum in the rat lung was 10^7 to 10^8 CFU, yet at the end of the study the mean number of CFU in the vehicle control animals was only in the range of 4.5 to 5.2 log$_{10}$/lung. As bacterial reduction is being measured in an experimental model which has a decreasing background of organisms, it is difficult to predict how the effective dose in the rodent will correlate with the effective dose in humans. For this reason both the 2- and 3-log$_{10}$ reductions in CFU were measured. These will ultimately be compared to the dose that demonstrates efficacy in the phase II and III clinical trials. Until then, we must be careful in extrapolation of these data for predicting the effective dose in humans.

Successful treatment of pulmonary infections, particularly those caused by *H. influenzae*, can typically be explained by the significant levels in tissue which are obtained with macrolides. The intrapulmonary pharmacokinetics of orally administered
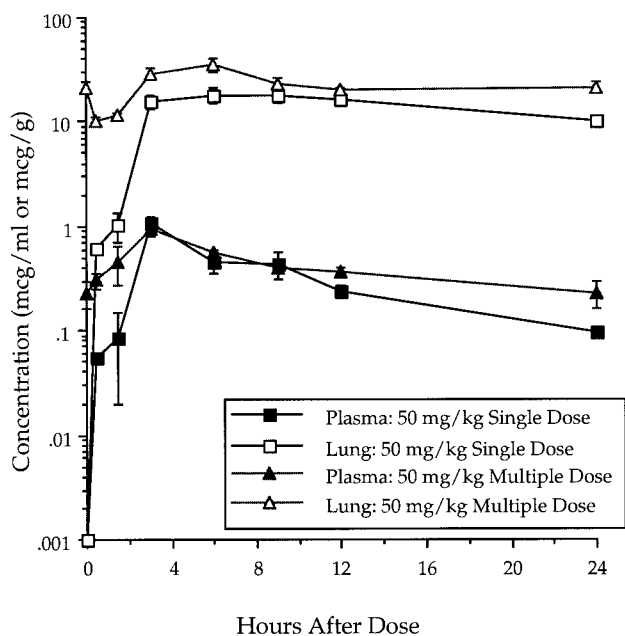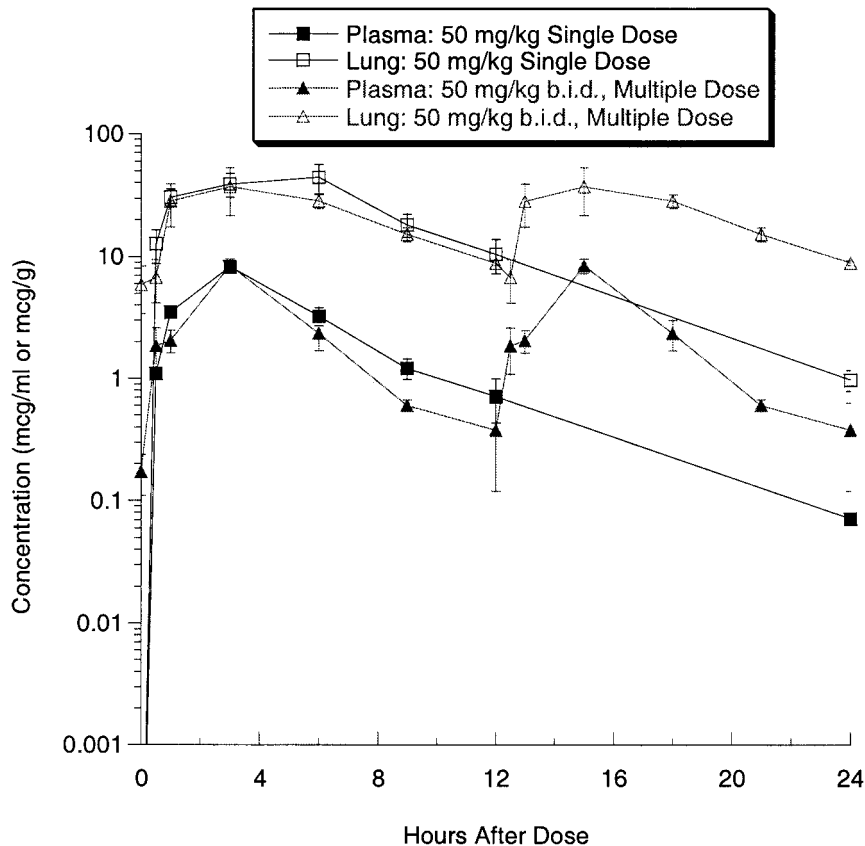
FIG. 4. Clarithromycin concentrations in plasma and lung after single and multiple 50-mg/kg oral dosing in rats. Error bars, standard errors.

clarithromycin (500 mg every 12 h) in humans revealed a range in lung epithelial lining fluid-to-plasma ratios of 13.3 to 27.6 based on clarithromycin concentrations obtained at 4 and 8 h post-final dosing (13). The actual concentrations of clarithromycin in the epithelial lining fluid were 29.3 and 72.1 μg/ml, respectively. The range in alveolar cell-to-plasma ratio was even higher at 98.7 to 229.9. The actual alveolar macrophage concentrations were 505.8 and 256.7 μg/ml, respectively (13). Healthy volunteers receiving five oral doses of azithromycin beginning with a dose of 500 mg on the first day and then 250

mg once daily for the next 4 days revealed lung epithelial lining fluid-to-plasma ratios of 12.6 and 24.2 based on assessments at 4 and 8 h post-final dosing, respectively (29). The actual concentrations of azithromycin in the epithelial lining fluid were 1.01 and 2.18 μg/ml, respectively. The alveolar cell-to-plasma ratios were 533.8 and 635.6. The actual alveolar macrophage concentrations were 42.7 and 57.2 μg/ml at 4 and 8 h, respectively (29).

Determining the drug levels in plasma and lung tissue may be useful for understanding efficacy studies in rats. The phar-

TABLE 6. In vitro susceptibilities of bacterial strains used in rodent models[a]

Bacterium	MIC (μg/ml) of:			
	ABT-773	Clarithromycin	Azithromycin	Penicillin
S. aureus ATCC 10649 (MLS$_B$^{s})	0.03	0.25		
S. pyogenes C203 (MLS$_B$^{s})	0.002	0.03		
L. monocytogenes 2526	0.03	0.12		
S. pneumoniae 6303 (MLS$_B$^{s})	0.001	0.008	0.03	
S. pneumoniae 6502 (MLS$_B$^{s} Pen^r)	0.001	0.004		2
S. pneumoniae 5649 (mefE, Pen^i)	0.06	4	8	0.5
S. pneumoniae 5654 (mefE, Pen^r)	0.06	4		2
S. pneumoniae 6396 (ermB, Pen^s)	0.015	>128	>128	0.06
S. pneumoniae 5669 (ermB, Pen^i)	0.015	>128		1
S. pneumoniae 5979 (ermB, Pen^r)	0.015	>128		4
H. influenzae 1435	0.5	2	1	
H. influenzae 3643	2	4	1	

[a] MICs were determined by the broth microdilution method for all isolates except for L. monocytogenes for which the NCCLS agar dilution method was used.

macokinetic profile of ABT-773, after single or multiple dosing at or near the dosage resulting in a 2-log reduction in lung bacterial burden for *H. influenzae* (25 mg/kg), produced a lung tissue-to-plasma AUC ratio of 29 (Table 5). The plasma and lung tissue AUCs were 12.03 and 350.5 μg · h/ml, respectively. This preliminary study demonstrated that the pharmacokinetics of ABT-773 includes a significant accumulation in lung tissue which is similar to those observed with other macrolides (23). Determining the free drug concentrations in lung epithelial lining fluid and intracellular concentrations extrapolated from alveolar macrophages in rats may be beneficial since these levels can be assessed in humans and may be important predictors of successful therapeutic outcomes (29). There is a need for sufficient drug levels in intracellular as well as extracellular compartments since some pathogens can be found in both (4). Studies have been planned to determine these levels for ABT-773 in both humans and rats.

The 50-mg/kg dose of azithromycin will generally produce a 2-$\log_{10}$ reduction in lung *Haemophilus* burden in rats. The single-dose C_{max} and AUC at 24 h (AUC_{24}) in plasma were 1.08 μg/ml and 8.6 μg · h/ml, respectively (Table 5). These values are approximately twofold higher than the reported C_{max} of 0.4 μg/ml and AUC_{24} of 3.4 μg · h/ml following a single 500-mg dose of azithromycin in humans (36). The clarithromycin concentrations in plasma following a 50-mg/kg single dose in rats (C_{max} of 8.2 μg/ml and AUC_{24} of 44.8 μg · h/ml) are also approximately twofold higher than the combined concentrations in plasma of clarithromycin and its bioactive metabolite (14-hydroxyclarithromycin not found in rodents), resulting in a C_{max} of 3.4 μg/ml and an AUC_{24} of 25 μg · h/ml (4, 36). The plasma elimination half-lives ($t_{1/2}$s) of both azithromycin and clarithromycin are shorter in rats than in humans.

When macrolide-susceptible strains of *S. pneumoniae* were tested, the ABT-773 MICs at which 90% of the strains are inhibited (MIC_{90}s) of 0.002 μg/ml were identical for penicillin-susceptible and penicillin-nonsusceptible strains (24). When evaluated against multidrug-resistant strains of *S. pneumoniae*, the ABT-773 MIC_{90}s are ≤0.008, 0.03, and 0.12 μg/ml for penicillin-susceptible, penicillin-intermediate, and penicillin-resistant strains, respectively (8). Reduced susceptibility to penicillin is an emerging clinical problem. In a recent surveillance study, 29.5% of *S. pneumoniae* isolates were not susceptible to penicillin (15). ABT-773 was compared to penicillin against *S. pneumoniae* using the lethal systemic mouse model. Penicillin had an ED_{50} of 22.2 mg/kg against a penicillin-susceptible strain (MIC = 0.06 μg/ml), but the range in ED_{50}s increased to 108.9 to >800 mg/kg for resistant strains with altered penicillin binding proteins (penicillin MIC ≥ 2 μg/ml). In contrast, ABT-773 was equally effective at treating the penicillin-susceptible and penicillin-resistant isolates. The efficacy results correlate with the in vitro antibacterial activity.

The efficacy of ABT-773 was consistently better than those of the comparative macrolide antibiotics against *S. pneumoniae*-induced infections in rodents caused by resistant (*mefE* or *ermB*) strains. Higher macrolide doses were required in mice and rats to achieve a similar therapeutic response against resistant strains than were required against susceptible strains. The susceptibilities to ABT-773 of the *S. pneumoniae* strains used in infections in experimental animal models were com-

parable to those of isolates evaluated in the 1997-to-1998 national surveillance study in which the MIC_{90} of ABT-773 for 1,601 strains was 0.03 μg/ml (8). Using the lethal systemic infection model caused by *S. pneumoniae* in mice, clarithromycin had an ED_{50} of 16.5 mg/kg against an MLS_B-susceptible strain, but the range in ED_{50}s increased to 123.8 to >200 mg/kg against *ermB* or *mefE* strains (clarithromycin MIC ≥ 4 μg/ml). ABT-773 was more effective at prolonging survival. It showed a range in ED_{50}s of 10.3 to 52.8 mg/kg against macrolide-susceptible, *ermB*, or *mefE* strains of *S. pneumoniae*. In rat lung infections, ABT-773 demonstrated >4-fold improved efficacy over azithromycin and clarithromycin, as measured by organ bacterial burden reduction, against the *mefE*-bearing *S. pneumoniae* (azithromycin, clarithromycin, and ABT-773 MICs = 8, 4, and 0.06 μg/ml, respectively). In lung infections associated with MLS_B-resistant *S. pneumoniae* (*ermB*), ABT-773 showed superior efficacy compared to the macrolide comparators (azithromycin, clarithromycin, and ABT-773 MICs = >128, >128, and 0.015 μg/ml, respectively). Thus, the efficacies of ABT-773 correlated well with the improved antibacterial activities of ABT-773 against macrolide-susceptible and -resistant strains in comparison with those of other macrolides.

The data presented in this paper demonstrate the utility of the new ketolide ABT-773 for treatment of susceptible and resistant respiratory tract pathogens. The efficacy achieved in systemic or pulmonary infections in rodents can be used as a baseline for future pharmacodynamic investigations in animals. Such studies can provide guidance in evaluating potential clinical doses and efficacy in humans.

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