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Activity of ABT-773 against *Mycobacterium avium* Complex in the Beige Mouse Model

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ABT-773, a new ketolide antimicrobial agent, was evaluated in comparison to clarithromycin (CLA) in vitro against *Mycobacterium avium* complex (MAC) and in a beige mouse model of disseminated MAC infection. The MICs at which 50 and 90% of the isolates tested were inhibited were 2 and 4 μg/ml, respectively, for CLA and 8 and 16 μg/ml, respectively, for ABT-773. Eight CLA-resistant isolates were found to be resistant to ABT-773 (MICs > 64 μg/ml). In the in vivo study mice were treated with ABT-773 (50, 100, and 200 mg/kg of body weight) or CLA (200 mg/kg). Both ABT-773 (100 and 200 mg/kg) and CLA significantly decreased the viable cell counts in spleens and lungs. ABT-773 (200 mg/kg) and CLA had similar activities in lungs, but the former was more active in spleens.

ABT-773 is a novel ketolide derived from erythromycin A (Z. Cao, R. Hammond, S. Pratt, A. Saiki, C. Lerner, and P. Zhong, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2135, 1999; Z. Ma, R. F. Clark, and Y. Or, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2133, 1999). It is active against macrolide-susceptible and -resistant respiratory pathogens (M. M. Neuhauser, J. L. Prause, R. Jung, N. Boyea, J. M. Hackleman, L. H. Danziger, and S. L. Pendland, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2139, 1999; P. Zhong, R. Hammond, Z. Cao, Y. Chen, D. A. Shortridge, A. Niulis, R. K. Flamm, and Y. Or, Abstr. 39th Intersci. Conf. Antimcicrob. Agents Chemother., abstr. 2134, 1999). Clarithromycin (CLA) and azithromycin have been used widely for the treatment and prevention of disseminated *Mycobacterium avium* complex (MAC) infection in patients with AIDS (3, 5, 7, 9, 11; P. Zhong et al., 39th ICAAC). They have also been used for the treatment of other nontuberculous mycobacterial infections (1, 2, 4, 8). The purpose of the present study was to evaluate the activity of ABT-773 compared with CLA against MAC in vitro and in the beige mouse model of disseminated MAC infection.

Drugs. ABT-773 and CLA were provided by Abbott Laboratories, Abbott Park, Ill. The drugs were dissolved in methanol for in vitro testing; for the in vivo dosing they were dissolved in absolute ethanol and subsequently diluted 1:5 (ABT-773 in phosphate-buffered saline and CLA in distilled water) to yield a 20% (vol/vol) ethanol solution. The drugs were freshly prepared each morning prior to administration.

Isolates. *M. avium* ATCC 49601 was obtained from the American Type Culture Collection, Manassas, Va. Clinical isolates of MAC were obtained from patients with AIDS at University Hospital, Syracuse, N.Y., and from Lowell Young (California Pacific Medical Center, San Francisco). The CLA-resistant isolates were provided by Abbott Laboratories.

Media. The organisms were grown in modified Middlebrook 7H10 broth (agar and malachite green omitted) (10) with 10% oleic acid-albumin-dextrose-catalase (OADC) enrichment and 0.05% Tween 80 on a rotary shaker at 37°C for 3 to 5 days prior to use.

Broth dilution method. Solutions of the drugs were sterilized by passage through a 0.22-μm-pore-size membrane filter and diluted in modified 7H10 broth to produce serial twofold dilutions from 64 to 0.125 μg/ml for ABT-773 and 16 to 0.125 μg/ml for CLA. These tubes and a control tube (containing no drug) were inoculated with a suspension of mycobacteria (0.1 ml of a 0.1-Klett unit/ml suspension containing about 5×10^5 organisms/ml) in 1.9 ml of broth to yield a final concentration of approximately 2.5×10^4 viable organisms/ml. The actual inocula were determined by titration on 7H10 agar plates with 5% OADC enrichment (range, 9.5×10^3 to 6.5×10^4 organisms/ml). The tubes were incubated on a rotary shaker at 37°C for 7 days prior to reading. The MIC was defined as the lowest concentration of antimicrobial agent yielding no visible turbidity. The isolates were studied in groups of five to eight organisms with ATCC 49601 as an internal control.

Infection study. A broth culture of ATCC 49601 was adjusted to 10 Klett units/ml containing approximately 5×10^7 viable organisms/ml. The actual inoculum was measured by titration on 7H10 agar plates. Eight-week-old male beige mice (C57BL/bJ bg^j/bg^j) obtained from Jackson Laboratories, Bar Harbor, Maine, were infected intravenously through a caudal vein with 2.2×10^6 viable *M. avium* ATCC 49601 organisms suspended in 0.2 ml of modified 7H10 broth. There were six mice per group. Treatment was started 7 days postinfection. ABT-773 (50, 100, and 200 mg/kg of body weight) and CLA (200 mg/kg) were administered by gavage 5 days/week for 4 weeks. An early control group (untreated) was sacrificed at the initiation of therapy, and a late control group was sacrificed at the end of therapy. Mice were euthanized by CO_2 inhalation 3 to 5 days after the administration of the last dose of drug. Spleens and right lungs were aseptically removed and were ground in a tissue homogenizer (Ideaworks Laboratory Devices, Syracuse, N.Y.). The number of viable organisms was determined by titration on 7H10 agar plates incubated at 37°C in ambient air for 3 weeks prior to counting.

Statistical evaluation. The viable cell counts were converted to logarithms, which were then evaluated by one- or two-variable analyses of variance. Statistically significant effects from the analyses of variance were further evaluated by the

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TABLE 1. Activity of ABT-773 compared to CLA in a beige mouse model of disseminated MAC infection

Treatment group[a]	Log_{10} CFU/organ (mean ± SD)	
	Spleen	Lung
Early controls (5)[b]	6.86 ± 0.12	4.15 ± 0.15
Late control (4)[c]	8.32 ± 0.10	6.69 ± 0.38
CLA, 200 mg/kg (5)[b]	5.26 ± 0.32	2.66 ± 0.18
ABT-773, 50 mg/kg (4)[d]	6.57 ± 0.10	3.92 ± 0.22
ABT-773, 100 mg/kg (6)	5.94 ± 0.21	3.04 ± 0.22
ABT-773, 200 mg/kg (5)[e]	4.69 ± 0.22	2.60 ± 0.50

[a] Values in parentheses are numbers of mice per group.
[b] One mouse was omitted due to technical error.
[c] One mouse died on day 26 after infection and one mouse was omitted due to technical error.
[d] Two mice died on days 22 and 32 after infection.
[e] One mouse died on day 15 after infection due to technical error.

Tukey honestly significant difference test (6) to make pair-wise comparisons among means.

In vitro susceptibilities of ABT-773 and CLA. The MICs at which 50 and 90% of the isolates tested were inhibited (MIC_{50} and MIC_{90}) were 8 and 16 μg/ml, respectively, for ABT-773 and 2 and 4 μg/ml, respectively, for CLA. The MIC range for ABT-773 was 0.25 to 32 μg/ml, and that for CLA was 0.25 to 8 μg/ml. Eight CLA-resistant clinical isolates (MICs $>$ 16 μg/ml) were found to be resistant to ABT-773 (MICs $>$ 64 μg/ml).

In vivo activity of ABT-773. ABT-773 had a dose-dependent effect on mycobacterial counts in spleens and lungs (Table 1). ABT-773 and CLA prevented an increase in the mycobacterial counts in spleens and lungs during the treatment period in comparison to that of the late control group. ABT-773 (100 and 200 mg/kg) and CLA reduced the cell counts in spleens and lungs compared to those in the early controls ($P < 0.05$). In the spleens, CLA was more active than ABT-773 at 100 mg/kg but less active than ABT-773 at 200 mg/kg ($P < 0.05$). In the lungs there was no difference between CLA and the two higher doses of ABT-773.

Although the in vitro activity of CLA against MAC was better than that of ABT-773, their activities were comparable in the beige mouse model of disseminated MAC infection. The promising activity of ABT-773 in the beige mouse model suggests that it may be useful for the treatment of MAC infection in humans and possibly for the treatment of infections caused by other nontuberculous mycobacteria. It was disappointing that CLA-resistant MAC was also resistant to ABT-773. Further evaluation of ABT-773 against nontuberculous mycobacteria, both in vitro and in vivo, would be useful to help define the potential clinical role for this agent.

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