ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1989, p. 1531–1534
0066-4804/89/091531-04$02.00/0

Vol. 33, No. 9

In Vitro and In Vivo Activities of Clarithromycin against *Mycobacterium avium*

PRABHAVATHI B. FERNANDES,†* DWIGHT J. HARDY, DONNA McDANIEL, CHARLES W. HANSON, AND ROBERT N. SWANSON

Anti-Infective Research, Abbott Laboratories, Abbott Park, Illinois 60064

Received 23 January 1989/Accepted 14 June 1989

There is no effective therapy to treat *Mycobacterium avium* complex infection in patients with acquired immune deficiency syndrome. Clarithromycin (A-56268; TE-031) is a new macrolide which is twofold more active than erythromycin against most aerobic bacteria. In addition, higher levels in serum and tissue are achieved with clarithromycin than with erythromycin. In this study, clarithromycin, erythromycin, difloxacin, temafloxacin, ciprofloxacin, rifampin, amikacin, and ethambutol were tested in vitro and in vivo against the *M. avium* complex. The MICs for 90% of strains tested were 4 μg/ml for clarithromycin, 64 μg/ml for erythromycin, 32 μg/ml for difloxacin, 8 μg/ml for temafloxacin, 4 μg/ml for ciprofloxacin, 4 μg/ml for rifampin, 32 μg/ml for amikacin, and 32 μg/ml for ethambutol. Beige mice were infected intravenously with 10^7 CFU of *M. avium* ATCC 25291. Treatment was started on day 6 after infection and was administered twice a day at 8-h intervals for 9 days. Clarithromycin was the most effective compound in these tests and was effective in reducing the viable bacterial counts in the spleen when it was administered subcutaneously or orally at a dose of 25 mg/kg. Amikacin was the only other compound which showed activity in vivo. The peak concentration in serum at which clarithromycin was active was approximately 1.0 μg/ml.

The treatment of *Mycobacterium avium* complex infections in immunocompromised patients is a problem since no agent is clearly effective against these bacteria (8, 9). We tested the activity of a new macrolide, clarithromycin (A-56268; TE-031) (2), against the *M. avium* complex in vitro and also in the beige mouse model (1, 4) to determine whether it could be useful in treating these infections.

MATERIALS AND METHODS

Bacterial strains. Ten strains of the *M. avium* complex were clinical isolates obtained from Christopher Papasian (Veterans Administration Medical Center, Kansas City, Mo.), and two strains, *M. avium* ATCC 25291 and *Mycobacterium intracellulare* ATCC 13950, were from the American Type Culture Collection (Rockville, Md.).

Antibacterial agents. Clarithromycin (A-62671; 14-hydroxy metabolite of clarithromycin), erythromycin, ciprofloxacin, difloxacin, and temafloxacin were prepared at Abbott Laboratories (Abbott Park, Ill.). Amikacin was obtained from Sigma Chemical Co. (St. Louis, Mo.), rifampin was obtained from United States Biochemical Corp. (Cleveland, Ohio), and ethambutol was obtained from Lederle Laboratories (Pearl River, N.Y.).

In vitro susceptibility tests. The in vitro susceptibility of the *M. avium* complex was determined by the agar dilution method (5). Mycobacteria were grown for 7 days in Middlebrook 7H9 broth supplemented with 0.05% (vol/vol) Tween 80–0.2% (wt/vol) glucose–10% (vol/vol) OADC (oleic acid, albumin, dextrose, and catalase; Difco Laboratories, Detroit, Mich.). This was then adjusted to match the turbidity of a McFarland 0.5 standard. This bacterial suspension, which contained 10^7 CFU/ml, as determined by duplicate plate counts, was inoculated with a Steers replicator onto Middlebrook 7H10 agar plates supplemented with 0.5% (vol/vol) glycerol–10% (vol/vol) OADC (Difco) containing twofold serial diltuions of the test antibacterial agents. Middlebrook 7H10 agar was used instead of Middlebrook 7H11 agar, because the casein hydrolysate in Middlebrook 7H11 agar is not necessary for the growth of *M. avium*. The plates were incubated in a humidified atmosphere for 5 days at 35°C. The plates were reincubated after they were read for up to 14 days, and the results were recorded again. The MIC was the lowest concentration of the antibacterial agent which allowed no growth.

Resistance frequency. The frequency of resistance to clarithromycin was determined by inoculating *M. avium* ATCC 25291 into Middlebrook 7H9 broth supplemented with 0.2% glycerol–0.2% glucose–10% OADC and incubating the culture for 24 days at 35°C. Serial 10-fold dilutions of this culture containing 6×10^9 CFU/ml were then prepared and

TABLE 1. In vitro activity of clarithromycin and reference compounds against the *M. avium* complex

Organism	MIC (μg/ml)[a]							
	CL	ERY	DIF	TEM	CIP	RIF	AMK	ETH
M. avium ATCC 25291	2	16	>64	32	16	4	8	16
M. avium complex								
3211	4	64	16	4	4	4	32	32
3212	2	64	16	8	1	1	8	32
3213	8	64	16	4	4	4	64	32
3214	2	16	16	8	2	1	4	16
3215	4	32	16	4	4	2	16	16
3216	2	32	32	16	4	2	16	16
3217	2	32	16	4	1	0.5	4	8
3218	2	32	16	4	2	4	32	32
3219	2	32	16	4	2	2	32	32
3220	2	32	32	8	4	8	32	32
M. intracellulare ATCC 13950	2	64	8	4	1	0.5	8	8

[a] Abbreviations: CL, clarithromycin; ERY, erythromycin; DIF, difloxacin; TEM, temafloxacin; CIP, ciprofloxacin; RIF, rifampin; AMK, amikacin; ETH, ethambutol.

* Corresponding author.
† Present address: The Squibb Institute for Medical Research, P.O. Box 4000, Princeton, NJ 08543-4000.

TABLE 2. Efficacy of clarithromycin and reference compounds against *M. avium* ATCC 25291

Compound	Log_{10} CFU (±SE) in spleens after treatment at[a]:	
	10 mg/kg	25 mg/kg
Clarithromycin	6.98 ± 0.13[b]	5.95 ± 0.05[b]
Temafloxacin	7.78 ± 0.10	7.92 ± 0.03
Ciprofloxacin	7.42 ± 0.15	7.76 ± 0.17
Difloxacin	7.84 ± 0.06	7.82 ± 0.06
Rifampin[c]	7.76 ± 0.52	7.93 ± 0.04

[a] Values are means of 10 mice. For untreated controls, the value was 7.80 ± 0.26 log_{10} CFU.
[b] Significantly different from untreated controls ($P < 0.05$). The viable bacterial count at the time of medication was 6.62 ± 0.14 CFU/ml.
[c] Rifampin was administered orally. Other compounds were administered subcutaneously.

inoculated onto Middlebrook 7H10 agar supplemented with 0.5% glycerol; 10% OADC; and two, four, or eight times the MIC of clarithromycin or erythromycin. The MICs of erythromycin and clarithromycin were 16 and 2 μg/ml, respectively, for *M. avium* ATCC 25291. One set of plates had no antibiotic and was the growth control. The plates were incubated at 35°C for 14 days, and the colonies were counted. The plates were reincubated for a total of 21 days, and the colonies were counted again to ensure that all resistant colonies were counted. All plates were examined with a magnifying glass in order to detect tiny colonies. All viable counts were performed on duplicate plates.

In vivo tests in the beige mouse model. The beige mouse model has been described by Gangadharam et al. (4). Beige mice (C57BL/6J *bgJ/bgJ*) were obtained from Jackson Laboratory (Bar Harbor, Maine) and were infected by intravenous injection via the tail vein with 10^7 CFU of *M. avium* ATCC 25291 in 0.2 ml of physiological saline (0.85% [wt/vol] sodium chloride; Abbott Laboratories). This strain was found to cause a persistent infection in the beige mice. Treatment was started on day 6 after infection. Groups of 10 mice each were treated with two dose levels of each compound. All compounds except rifampin and clarithromycin were administered subcutaneously. Rifampin was administered orally, and clarithromycin was administered orally and subcutaneously. Rifampin was administered orally because it is well absorbed by this route and has been administered by this route previously (6). Except for amikacin, rifampin, and ethambutol, the doses were selected to obtain concentrations in the blood which were similar to the achievable levels in blood in humans. Doses of amikacin (L. Kesavalu, P. R. J. Gangadharam, V. K. Perumal, N. R. Podapati, and M. D. Iseman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, U67, p. 137), rifampin, and ethambutol (6) were chosen on the basis of those used in previous studies. The mice were treated daily for 9 days. The daily dose was divided into two equal doses, and the two parts were administered 8 h apart. A group of 10 mice was left untreated as the infection control. The mice were killed 18 h after the last treatment; and the spleens were aseptically collected, homogenized, and cultured quantitatively. The geometric mean of the viable bacterial counts (CFU per organ) was calculated for each group.

Statistical evaluation. A one-way analysis of variance model was used to evaluate the overall effect of the treatment groups. The Fisher least-significant-difference test was then used to compare the results that were obtained with clarithromycin and the reference compounds with those obtained from the untreated controls. In addition, results were compared both between and within the groups that were treated by different routes of administration.

Pharmacokinetic studies. The levels of the compounds in blood were determined in C57BL/6J mice (Jackson Laboratory). Although these mice do not have a deficient immune system like the beige mice, they are similar to the beige mice in other respects. They were used instead of beige mice for the pharmacokinetic studies because of the limited availability of beige mice. The dose of each antibacterial agent and the route of administration for the pharmacokinetic studies were the same as those in the treatment experiments, except that a single dose was administered. Blood and spleens were collected from groups of five mice each at 0.5, 1, 2, 3, 6, and 24 h. The concentrations of the antibacterial agents in sera and spleens were determined by bioassay. The bioassay for clarithromycin has been described previously (3). Blood and spleens were collected from groups of five mice each at 0.5, 1, 2, 3, 6, and 24 h on day 5 of treatment in order to determine whether there was a buildup of clarithromycin in sera and spleens after repeated administration.

RESULTS

In vitro activity. The MICs of macrolides, fluoroquinolones, and reference compounds for the *M. avium* complex are given in Table 1. The colonies were larger at 21 days than at 5 days; otherwise, the results were the same at 5 and 21 days after incubation. Clarithromycin was 8- to 32-fold more

TABLE 3. Efficacy of clarithromycin and reference compounds against *M. avium* ATCC 25291

Compound	Route	Log_{10} CFU (±SE) in spleens after treatment at[a]:			
		10 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Clarithromycin	Oral			5.87 ± 0.04[b]	5.02 ± 0.27[b]
Clarithromycin	s.c.[c]	6.56 ± 0.03[b]	6.20 ± 0.08[b]		
Erythromycin	s.c.	7.93 ± 0.1	7.86 ± 0.12		
Amikacin	s.c.	6.91 ± 0.03[b]	6.60 ± 0.13[b]		
Clarithromycin-amikacin[d]	s.c.	6.78 ± 0.04[b]	6.24 ± 0.09[b]		
Amikacin-ethambutol-rifampin[e]	s.c.	6.52 ± 0.07[b]			
	Oral				
	Oral				

[a] Values are means of 10 mice. For untreated controls, the value was 7.81 ± 0.30 log_{10} CFU.
[b] Significantly different from untreated controls ($P < 0.05$). The viable bacterial count at the time of medication was 6.68 ± 0.18 CFU/ml.
[c] s.c., Subcutaneous.
[d] For the 10- and 25-mg/kg doses, each compound was used at a dose of 10 and 25 mg/kg, respectively.
[e] Amikacin, ethambutol, and rifampin were used at 25, 10, and 10 mg/kg, respectively.

TABLE 4. Comparison of the efficacies of clarithromycin when administered orally or subcutaneously

Route	Log_{10} CFU (±SE) in spleens after treatment at[a]:		
	6.2 mg/kg	12.5 mg/kg	25 mg/kg
Oral	7.63 ± 0.09	7.38 ± 0.06[b]	6.87 ± 0.12[b]
s.c.[c]	7.29 ± 0.15	7.14 ± 0.15	6.24 ± 0.05[b]

[a] Values are means of 10 mice. For untreated controls, the value was 7.92 ± 0.20 log_{10} CFU.
[b] Significantly different from untreated controls ($P < 0.05$). The viable bacterial count at the time of medication was 6.39 ± 0.13 CFU/ml.
[c] s.c., Subcutaneous.

active than erythromycin. Ciprofloxacin was the most active fluoroquinolone.

Resistance frequency. No *M. avium* colonies were selected on plates containing two, four, or eight times the MIC of clarithromycin; and the resistance frequency of *M. avium* ATCC 25291 was calculated to be $<1.8 \times 10^{-10}$ when tested with the three concentrations of clarithromycin. One resistant colony was selected at both four and eight times the MIC of erythromycin, and no colonies were selected at two times the MIC of erythromycin. The resistant colony was susceptible to >128 μg/ml, whereas the parent organism was susceptible to 16 μg/ml. The plates were incubated for as long as 21 days in order to determine whether any resistant colonies would grow after prolonged incubation. No other resistant colonies were found.

The resistance frequency for erythromycin was calculated to be 1.8×10^{-10} at four and eight times the MIC and $<1.8 \times 10^{-10}$ at two times the MIC.

In vivo activity. In the first experiment, clarithromycin, temafloxacin, ciprofloxacin, difloxacin, and rifampin were tested at 10 and 25 mg/kg. All compounds except rifampin were administered subcutaneously. Rifampin was administered orally by gavage. The viable bacterial counts recovered from the spleens are given in Table 2. At 10 and 25 mg/kg, clarithromycin was found to have a significantly different effect from those of all other treatment groups and decreased the viable bacterial counts by approximately 2 log_{10} CFU. The fluoroquinolones and rifampin were not effective when tested as single agents.

In the second experiment, clarithromycin was administered orally by gavage at 50 and 100 mg/kg and subcutaneously at 10 and 25 mg/kg. Erythromycin and amikacin were tested subcutaneously at 10 and 25 mg/kg. Two combination treatments, clarithromycin-amikacin and amikacin-ethambutol-rifampin, were tested. The results are given in Table 3. Clarithromycin was effective in reducing the bacterial counts after subcutaneous and oral therapies. The oral route of administration was as effective as the subcutaneous route for clarithromycin. When administered orally, amikacin was less effective than clarithromycin in reducing bacterial counts in the spleens. The number of bacteria recovered from the spleens of mice treated with combinations of amikacin and other drugs was similar to the number of bacteria recovered from mice treated with amikacin alone. The lowest dose at which clarithromycin was consistently effective in reducing viable bacterial counts by 1 log_{10} CFU/ml was 25 mg/kg when administered subcutaneously or orally (Table 4).

Pharmacokinetics in mice. The concentrations of clarithromycin and test compounds in sera and spleens are given in Table 5. Clarithromycin had a longer half-life in the sera and spleens than erythromycin did. Clarithromycin concentrations in the spleens were approximately 10 times those of the concentrations in sera. There was only a slight increase in the concentrations of clarithromycin in sera and spleens after 5 days of continuous therapy (Table 6). The somewhat lower concentrations of clarithromycin in sera and spleens could be the result of enhanced metabolism after repeated administration.

DISCUSSION

Clarithromycin is a new macrolide which is well absorbed orally and has a longer half-life in serum than erythromycin does. It is twofold more active than erythromycin against most bacteria in the macrolide spectrum (2). Therefore, it was surprising to find in this study that it was 8- to 32-fold more active than erythromycin in vitro against the *M. avium*

TABLE 5. Pharmacokinetics of clarithromycin and other antibacterial agents tested against *M. avium*

Compound (dose [mg/kg])	Route	Site	Concn (μg/ml) at the following times (h):						$t_{1/2}$ (h)[a]	AUC (μg · h/ml)[b]
			0.5	1	2	3	6	24		
Clarithromycin										
10	s.c.[c]	Serum	1.1	0.8	0.3	0.2	0	0	0.9	1.2
		Spleen	14.1	9.6	4.6	2.3	0	0	0.9	16.3
25	s.c.	Serum	2.4	2.0	0.9	0.7	0	0	1.4	3.9
		Spleen	24.5	21.2	12.7	6.1	1	0	1.1	50.8
25	Oral	Serum	0.9	0.9	0.4	0.2	0	0	1.1	1.5
		Spleen	8.6	8.4	7.4	3.6	0	0	2.0	20.8
50	Oral	Serum	2.6	2.0	1.8	0.9	0.2	0	1.3	6.9
		Spleen	21.2	22.2	16.5	7.9	1.7	0	1.4	66.3
Erythromycin (25)	s.c.	Serum	3.7	2.3	0.4	0.1	0	0	0.5	2.4
		Spleen	9	6.4	1	0	0	0	0.5	5.8
Ciprofloxacin (25)	s.c.	Serum	3.9	3.1	2.2	0.7	0.2	0	1.4	7.5
		Spleen	17.8	11.9	7.0	1.8	0	0	0.8	19.5
Difloxacin (25)	s.c.	Serum	4.2	4.3	2.6	2.4	1.8	1.9	24.9	>45
		Spleen	9.4	7.3	6.0	5.6	1.9	5.4	>24	>108
Temafloxacin (25)	s.c.	Serum	3.5	1.4	2.9	1	0.2	0	1.9	8.9
		Spleen	10.1	13.3	2.8	2	0	0	0.9	16.7

[a] $t_{1/2}$, Half-life.
[b] AUC, Area under the concentration-time curve.
[c] s.c., Subcutaneous.

TABLE 6. Pharmacokinetics of clarithromycin after 5 days of therapy[a]

Route	Site	Concn (μg/ml) at the following times (h):						$t_{1/2}$ (h)[b]	AUC (μg · h/ml)[c]
		0.5	1	2	3	6	24		
s.c.[d]	Serum	1.5	1.1	0.7	0.4	0	0	1.4	2.4
	Spleen	17.2	13.4	11.0	6.0	0	0	1.7	33.0
Oral	Serum	0.5	0.5	0.4	0.1	0	0	1.1	1.0
	Spleen	6.0	6.3	4.5	2.2	0	0	1.7	13.8

[a] Clarithromycin was used at a dose of 25 mg/kg.
[b] $t_{1/2}$, Half-life.
[c] AUC, Area under the concentration-time curve.
[d] s.c., Subcutaneous.

complex. The reason for this difference in susceptibility may be related to the apparent log P values of the two macrolides. The apparent log P of erythromycin measured at pH 7.4 is 1.2, whereas the log P of clarithromycin measured under the same conditions is 1.7 (L. Freiberg, Abbott Laboratories, unpublished observations). Since clarithromycin is more lipophilic, it may be able to penetrate the lipid coat of mycobacteria more readily than erythromycin can (7).

Among the compounds that were tested in this study, clarithromycin was the most active compound in vivo in three experiments. The in vivo activity of clarithromycin against *M. avium* may be related to the pharmacokinetic properties of the compound coupled with its in vitro activity and lower resistance frequency. Clarithromycin has been reported to achieve high concentrations in tissue (2) and to have high intracellular concentrations (T. Suma, H. Yoshida, K. Fukushima, and H. Kobayashi, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother. abstr. no. 417, 1986). Since clarithromycin is more acid stable than erythromycin, it is possible that it would remain active for a longer period of time than erythromycin in the acidic environment of the phagolysosome.

Combination therapy is generally used against mycobacteria to prevent resistance development. In our studies, the resistance frequency was $<1.8 \times 10^{-10}$ for *M. avium* ATCC 25291 when tested with clarithromycin. The reason for the lack of efficacy of erythromycin when used as a single drug could be because the concentration of erythromycin in the blood and tissues is lower than twice the MIC for *M. avium*. Further work needs to be done in evaluating the activity of clarithromycin when used with other antimycobacterial drugs, such as clofazimine. Other new macrolides have also been reported to have activity against *M. avium* (8). Since clarithromycin as a single agent was shown to have in vitro and in vivo activities against the *M. avium* complex, this compound should be tested either as a single drug or in combination with clofazimine and other antimycobacterial agents in patients infected with the *M. avium* complex.

ACKNOWLEDGMENTS

We thank our co-workers in the microbiology team who gave us technical assistance during these studies and R. Rode for statistical evaluation.

LITERATURE CITED

1. **Bertram, M. A., C. B. Inderlied, S. Yadegar, P. Kolanoski, J. K. Yamada, and L. S. Young.** 1986. Confirmation of the beige mouse model for the study of disseminated infection with *Mycobacterium avium* complex. J. Infect. Dis. **154**:194–195.
2. **Fernandes, P. B., R. Bailer, R. Swanson, C. W. Hanson, E. McDonald, N. Ramer, D. Hardy, N. Shipkowitz, R. R. Bower, and E. Gade.** 1986. In vitro and in vivo evaluation of A-56268 (TE-031), a new macrolide. Antimicrob. Agents Chemother. **30**:865–873.
3. **Fernandes, P. B., N. Ramer, R. A. Rode, and L. Freiberg.** 1988. Bioassay for A-56268 (TE-031) and identification of its major metabolite, 14-hydroxy-6-*O*-methyl erythromycin. Eur. J. Clin. Microbiol. Infect. Dis. **7**:73–76.
4. **Gangadharam, P. R., C. K. Edwards III, P. S. Murthy, and P. F. Pratt.** 1983. An acute infection model for *Mycobacterium intracellulare* disease using beige mice: preliminary results. Am. Rev. Respir. Dis. **127**:648–649.
5. **Inderlied, C. B., L. S. Young, and J. K. Yamada.** 1987. Determination of in vitro susceptibility of *Mycobacterium avium* complex isolates to antimycobacterial agents by various methods. Antimicrob. Agents Chemother. **31**:1697–1702.
6. **Kuze, F.** 1984. Experimental chemotherapy in chronic *Mycobacterium avium-intracellulare* infection of mice. Am. Rev. Respir. Dis. **129**:453–459.
7. **Rastogi, N., C. Frehel, A. Ryter, H. Ohayon, M. Lesourd, and H. L. David.** 1981. Multiple drug resistance in *Mycobacterium avium*: is the wall architecture responsible for the exclusion of antimicrobial agents? Antimicrob. Agents Chemother. **20**:666–677.
8. **Young, L. S.** 1988. *Mycobacterium avium* complex infection. J. Infect. Dis. **157**:863–867.
9. **Young, L. S., C. Inderlied, O. G. Berlin, and M. S. Gottlieb.** 1986. Mycobacterial infections in AIDS patients, with an emphasis on the *Mycobacterium avium* complex. Rev. Infect. Dis. **8**:1024–1033.