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In Vitro Activity of Roxithromycin against 16 Species of Atypical Mycobacteria and Effect of pH on Its Radiometric MICs

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The antimycobacterial spectrum of roxithromycin, a semisynthetic 14-membered ring macrolide, was determined against 28 strains belonging to 16 species of atypical mycobacteria by measuring radiometric MICs by BACTEC methodology at two different pH values, i.e., 6.8 and 7.4. The MICs obtained at pH 7.4 were 1 to 2 dilutions lower or more than those obtained at pH 6.8 for some of the species. Roxithromycin possessed promising MICs against such potential pathogens as the *Mycobacterium avium* complex, *M. scrofulaceum*, *M. szulgai*, *M. malmoense*, *M. xenopi*, *M. marinum*, *M. kansasii*, and rare pathogens like *M. chelonei* subsp. *chelonei* and *M. chelonei* subsp. *abscessus* but not against *M. simiae*. Roxithromycin showed lower MICs against *M. fortuitum* var. *peregrinum* than *M. fortuitum* var. *fortuitum*.

Because of its rapid acid degradation (4) and relatively low plasma levels (5), erythromycin A has not been widely used as an antimycobacterial agent. By modifying the ketone at the C-9 position, erythromycin A was stabilized but its potent antimicrobial activity was retained and its pharmacokinetic properties were simultaneously improved (4). Roxithromycin (RU-28965), which is the 9-[*O*-(2-methoxy ethoxy)methyl]-oxime of erythromycin A, was selected as the potential candidate with the best therapeutic index among many other oxime derivatives synthesized (4). If the pharmacokinetic properties of roxithromycin are compared with those of clarithromycin (6-*O*-methylerythromycin A), roxithromycin is found to be characterized by higher concentrations in the serum (5). Indeed, the peak serum drug level of roxithromycin measured 10.8 μg/ml (time to peak, 1.5 h) after administration of a single dose of 300 mg, whereas the peak serum drug level of clarithromycin was 4.4 μg/ml (time to peak, 1.7 h) after administration of a dose of 1,200 mg (5).

On the basis of the above considerations and our previous reports suggesting a privileged role for the newer macrolide clarithromycin against mycobacterial infections (10–12), we thought it desirable to determine the antimycobacterial spectrum of roxithromycin. Although many reports have described MICs of roxithromycin against the organisms of the *Mycobacterium avium* complex (1, 7, 8), the antimycobacterial spectrum of this drug has not yet been investigated. Since recent reports have shown a higher activity of clarithromycin at an alkaline pH (3, 10), we decided to determine the MICs of roxithromycin at pHs 6.8 and 7.4. pH 6.8 was taken as the standard pH used for drug screening in mycobacteria, whereas pH 7.4 was used on the basis of the physiological pH prevailing in the plasma.

The various mycobacterial species used in this investigation (Table 1) were grown in complete 7H9 broth (supplemented with Middlebrook ADC enrichment; Difco Laboratories, Detroit, Mich.) containing 0.05% (vol/vol) Tween 80 to avoid clumping at 37°C and harvested at the mid-logarithmic growth phase at an optical density of 0.15 (measured at 650 nm with a Coleman Junior II spectrophotometer) which corresponded to about 10^8 CFU/ml.

The radiometric determination of MICs was performed by using the BACTEC 460-TB apparatus (Becton Dickinson, Towson, Md.) reported earlier (3, 9, 10, 12, 13). Briefly, bacterial growth was measured in a confined atmosphere as a function of the ability of the bacteria to catabolize ^{14}C-labeled palmitic acid in the 7H12 broth and by automatically measuring the $^{14}CO_2$ released. The growth of bacteria was represented as a numerical value called the growth index (GI), which ranged from 1 to 999. Mycobacterial growth in this system is dependent on the standardization of the initial bacterial inoculum, and because of the more rapid growth of *M. avium* and some other rapid growers compared with *M. tuberculosis* in the BACTEC system, the initial bacterial inoculum added to the BACTEC vials depended on the species studied.

After a preculture of a strain in an initial BACTEC vial to a GI of 500, the drug-containing vials were inoculated with 0.1 ml of the preculture used directly in the case of *M. xenopi*, whereas they were inoculated with 0.1 ml of a preculture diluted 1:10 in the case of all other atypical mycobacteria except *M. avium*, *M. fortuitum*, and *M. chelonei* (which were inoculated with 0.1 ml of a preculture diluted 1:100). The change in daily GI (ΔGI) of the above drug-containing vials was compared with that of a control vial which was initially inoculated with 100-times-fewer bacteria in the absence of the drug. Under these conditions, the MIC was interpreted once the GI in the 1:100-diluted control reached a value of 30 or more and was defined as the minimal drug concentration resulting in a ΔGI in the drug-containing sample lower than that of the control.

All of the MICs were determined in parallel at pHs 6.8 and 7.4. For pH 6.8, the medium used was commercially available BACTEC 12B broth (Becton Dickinson), whereas in the latter case, the pH in the 12B vials was adjusted to 7.4 by

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TABLE 1. In vitro activity of roxithromycin against 16 species of atypical mycobacteria and effect of pH on its radiometric MICs

Pathogen type and species	MIC (μg/ml)[a]	
	pH 6.8[b]	pH 7.4[c]
Potential pathogens		
M. avium CIPT 140310002	1.0	0.5
Clinical isolate 89-0733	2.0	1.0
Clinical isolate 90-0827	8.0	2.0
Clinical isolate 91-0788	8.0	2.0
M. scrofulaceum ATCC 19981	1.0	0.5
M. simiae ATCC 25275	32.0	8.0
AIDS isolate 91-0198	32.0	16.0
M. szulgai NCTC 10831	4.0	1.0
M. malmoense ATCC 29571	2.0	0.5
M. xenopi ATCC 19970	0.5	0.25
M. marinum ATCC 927	0.5	0.5
M. kansasii ATCC 12478	1.0	0.5
Rare pathogens		
M. gordonae ATCC 14470	0.5	<0.25
M. terrae ATCC 15755	2.0	1.0
M. triviale ATCC 23292	1.0	0.5
M. gastri ATCC 15754	1.0	0.25
M. chelonei subsp. *chelonei* NCTC 946	8.0	1.0
M. chelonei subsp. *chelonei* CIPT 140420005	2.0	1.0
Clinical isolate 81-0402	2.0	1.0
Clinical isolate 92-0592	1.0	0.5
M. chelonei subsp. *abscessus* clinical isolate 92-0801	8.0	2.0
M. fortuitum var. *fortuitum* ATCC 6841	64.0	16.0
M. fortuitum var. *fortuitum* CIPT 140410002	16.0	4.0
Clinical isolate 92-0542	64.0	16.0
M. fortuitum var. *peregrinum* ATCC 14467	2.0	1.0
Clinical isolate 92-0469	16.0	4.0
Clinical isolate 92-0580	8.0	2.0

[a] All of the MICs were determined radiometrically. In the case of *M. xenopi*, the drug-containing vials were inoculated with 0.1 ml of a preculture grown to a BACTEC GI of about 500, whereas in all other cases, they were inoculated with 0.1 ml of a 1:10-diluted culture (except *M. avium*, *M. fortuitum*, and *M. chelonei*, which were inoculated with a 1:100-diluted preculture). The values in each case were compared with those of a control vial containing an inoculum that had been further diluted 1:100.

[b] BACTEC 12B medium with a pH of 6.8 was used.

[c] Medium pH was fixed at 7.4 by using NaOH.

adding 0.1 ml of a standardized NaOH solution as previously reported (10). Changing the pH to 7.4 from the routine pH of 6.8 did not significantly affect the growth kinetics of the bacteria studied in the respective controls, and nearly all of the MICs were interpretable within a similar time period (4 to 7 days depending on the species studied). The pH of 7.4 was found to be stable in parallel uninoculated controls during the time allotted for experimentation. The roxithromycin concentrations screened radiometrically consisted of two overlapping ranges of 0.25, 0.5, 1, 2, 4, 8, 16, and 32 μg/ml at pH 7.4 and 1, 2, 4, 8, 16, 32, and 64 μg/ml at pH 6.8.

Roxithromycin (Roussel-Uclaf, Paris, France) and the BACTEC 460-TB apparatus (Becton-Dickinson) used in this investigation were kindly provided by the manufacturers.

Table 1 illustrates the antimycobacterial spectrum of roxithromycin against 28 strains belonging to 16 species of mycobacteria determined radiometrically by establishing MICs at both pH 6.8 and pH 7.4. The MICs obtained at pH 7.4 were 1 to 2 dilutions lower or more than those obtained at pH 6.8 in most of the species. The drug had MICs below its peak serum level (10.8 μg/ml) against 21 of 28 strains (representing 13 of 16 species) at pH 6.8 and 24 of 28 strains (representing 15 of 16 species) at pH 7.4. As found recently in the case of clarithromycin (10), roxithromycin did not show promising MICs against *M. simiae* at either pH tested.

If the MICs in Table 1 are compared with those recently reported for clarithromycin (10), it can be concluded that roxithromycin possessed an antimycobacterial spectrum similar to that of the latter, with promising MICs against such potential pathogens as the *M. avium* complex, *M. scrofulaceum*, *M. szulgai*, *M. malmoense*, *M. xenopi*, *M. marinum*, *M. kansasii*, and rare pathogens like *M. chelonei* subsp. *chelonei* and *M. chelonei* subsp. *abscessus*. Roxithromycin showed lower MICs against *M. fortuitum* var. *peregrinum* than against *M. fortuitum* var. *fortuitum*.

Macrolides, in general, concentrate inside phagocytes from 2- to more than 10-fold in the case of erythromycin and clarithromycin (5, 6, 14). In a model of *Staphylococcus aureus*-infected J-774 macrophages, roxithromycin has been reported to concentrate more than 20-fold (14). Thus, a comparison of the roxithromycin MICs (Table 1) with projected intracellular concentrations suggests that, like clarithromycin (10–12), roxithromycin may also possess promising activity for the treatment of infections caused by a variety of potentially pathogenic (or opportunistic) mycobacteria. The efficacy of clarithromycin in eliminating *M. avium* from the blood of patients with AIDS has been proposed to be associated with its high inhibitory activity at pH 7.4 (3). Considering that the intracellular accumulation of roxithromycin is higher under nonacidic than acidic conditions (14) and that vesicles containing living, virulent *M. avium* in experimentally infected human macrophages are indeed not acidic (2), further studies dealing with extracellular and intracellular activity of roxithromycin used alone and in association with other drugs are in progress in our laboratory.

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