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In Vitro Susceptibility of *Mycobacterium ulcerans* to Clarithromycin

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Buruli ulcer (BU), caused by *Mycobacterium ulcerans*, was recently recognized by the World Health Organization as an important emerging disease. While antimycobacterial therapy is often effective for the earliest nodular or ulcerative lesions, medical management of BU lesions in patients presenting for treatment is usually disappointing, leaving wide surgical excision the only alternative. Advanced ulcerated lesions of BU rarely respond to antimycobacterial agents; however, perioperative administration of such drugs may prevent relapses or disseminated infections. Clarithromycin possesses strong activity in vitro and in vivo against most nontuberculous mycobacteria. In this study we determined the antimycobacterial activity of this drug in vitro against 46 strains of *M. ulcerans* isolated from 11 countries. The MIC of clarithromycin was determined at pH 6.6 (on 7H11 agar) and at pH 7.4 (on Mueller-Hinton agar). The MICs ranged from 0.125 to 2 μg/ml at pH 6.6 and from <0.125 to 0.5 μg/ml at pH 7.4. For the majority of the strains, geographic origin did not play a significant role. Thirty-eight strains (83%) were inhibited by 0.5 μg/ml at pH 7.4. These MICs are below peak therapeutic concentrations of clarithromycin obtainable in blood. These results suggest that clarithromycin is a promising drug both for the treatment of early lesions of *M. ulcerans* and for the prevention of hematogenous dissemination of the etiologic agent during and after surgery. Studies should be initiated to evaluate the effects of clarithromycin in combination with ethambutol and rifampin on *M. ulcerans* both in vitro and in experimentally infected mice. Multidrug regimens containing clarithromycin may also help control the secondary bacterial infections sometimes seen in BU patients, most importantly osteomyelitis.

Mycobacterium ulcerans causes necrotizing, relatively nonpainful lesions known variously as Buruli ulcer (BU), Bairnsdale ulcer, or more precisely, *M. ulcerans* infection. BU was recently recognized by the World Health Organization as an important emerging disease. The disease has been reported in many countries, mostly tropical, in Africa, North America (Mexico), South America, Southeast Asia, and Australia. Recent reports have suggested increased incidences of BU in, for example, some areas of Benin (21), Australia (13), and Côte d'Ivoire (24).

Medical treatment of these ulcers is usually disappointing, leaving wide surgical excision followed by skin grafting the only alternative (4). Preulcerative nodular lesions and early small ulcerated lesions can be effectively treated by excision and primary closure, by rifampin alone, or possibly by heating at 40°C without prior excision (26). Rifampin, however, is usually not effective against advanced ulcers. Even though currently the best therapeutic approach is surgery, it is possible that postsurgical antimycobacterial treatment would prevent relapses or metastatic infections. Disseminated infections with involvement of bone and cartilage have been observed (3, 10).

Among the new macrolides, clarithromycin possesses strong activity in vitro and in vivo against most nontuberculous mycobacteria. Clarithromycin is effective for the treatment of infections caused by *Mycobacterium avium* complex in AIDS patients (8, 11), *Mycobacterium genavense* (5) and *Mycobacterium haemophilum* (22) infections, lymphadenitis caused by nontuberculous mycobacteria (36), and *Mycobacterium marinum* infections in both human immunodeficiency virus-seronegative and -seropositive patients (6). Numerous investigations have shown clarithromycin to be active in the treatment of infections caused by rapidly growing mycobacteria (7, 25, 28, 30, 37, 38), and it is active in vitro and in vivo against *M. avium* (12) and *Mycobacterium leprae* (14).

Several reports suggest that the susceptibilities of some mycobacterial species to clarithromycin depend on the pH of the medium and that the MIC of clarithromycin for these species is lower at pH 7.4 than at pH 6.6 (20, 32).

Based on the reported efficacy of clarithromycin for the treatment of leprosy and some other nontuberculous mycobacterial infections, we determined the antimycobacterial activities of this drug in vitro at pH 6.6 and at pH 7.4 against strains of *M. ulcerans* of different geographic origins. We chose to assess the MIC at pH 6.6 and 7.4 because pH 6.6 is the standard pH of 7H11 medium, which is commonly used for drug sensitivity tests and determination of the MIC for mycobacteria (19), and because the physiological pH of plasma is 7.4.

MATERIALS AND METHODS

Antimicrobial agent. Clarithromycin was kindly provided by Abbott Laboratories Ltd. (Queenborough, Kent, England). The stock solution (2 mg/ml) and working solutions were prepared as described by Mor and Heifets (27).

MIC determination. Because the antimycobacterial efficacy of clarithromycin depends on the pH of the medium, the MIC of clarithromycin was determined on two different media: 7H11 agar (pH 6.6) and Mueller-Hinton agar (pH 7.4) supplemented with 10% (vol/vol) OADC (oleic acid, albumin, dextrose, and catalase; Difco Laboratories, Detroit, Mich.) (20, 32). Both media contained twofold dilutions of clarithromycin from 0.125 to 4 μg/ml. Media without antibiotics added served as controls. The inocula were 0.1 ml of 10^{-1} and 10^{-3} dilutions of bacterial suspensions of 1 mg/ml in phosphate-buffered saline. The tubes were incubated at 33°C and were read after 28 and 42 days of incubation. The MIC was defined as the lowest concentration of clarithromycin inhibiting ≥99% of the bacterial population in the 10^{-1} dilution. Only tests with growth in the tubes inoculated with the 10^{-3} dilution were considered valid.

Bacterial strains. A total of 46 *M. ulcerans* strains isolated from patients from 11 countries were studied (see Table 1). One ATCC reference strain, ATCC

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TABLE 1. MICs of clarithromycin against 46 isolates of *M. ulcerans* from 11 different countries

Origin (*n*) of isolates	pH	No. tested	Cumulative % of strains inhibited at the following MIC (μg/ml):						
			<0.125	0.125	0.25	0.50	1	2	4
Australia (12)	6.6	12				42	92	100	
	7.4	8	88			100			
Benin (9)	6.6	7		43	57	86	100		
	7.4	6	33		100				
Côte d'Ivoire (5)	6.6	5			20	100			
	7.4	4		25	75	100			
Democratic Republic of Congo (8)	6.6	8		25	63	88	100		
	7.4	4	20		100				
Ghana (4)	6.6	4			100				
	7.4	4		25	75	100			
Angola (1)	6.6	1			100				
	7.4	1			100				
Togo (1)	6.6	1				100			
	7.4	1	100						
French Guiana (1)	6.6	1				100			
	7.4	1		100					
Mexico (1)	6.6	1		100					
Papua New Guinea (3)	6.6	3			67	100			
Malaysia (1)	6.6	1						100	

19423, from Australia, was studied. A total of 41 strains were isolated at the Institute of Tropical Medicine from tissue fragments transported to Antwerp (31), and 5 strains were obtained from other laboratories: 4 (1 from Papua New Guinea and 3 from Malaysia) were contributed by K. Jackson (Fairfield Hospital, Melbourne, Australia), and 1 (from French Guiana) was contributed by V. Vincent, Institut Pasteur, Paris, France.

Fresh subcultures were made on tubes of Löwenstein-Jensen medium.

RESULTS

As indicated in Table 1, the MICs of clarithromycin for the 44 *M. ulcerans* strains tested on 7H11 medium (pH 6.6) ranged from 0.125 to 2 μg/ml and the MICs for the 29 strains tested on Mueller-Hinton medium (pH 7.4) ranged from <0.125 to 0.5 μg/ml. All tested strains were inhibited by 2 μg of clarithromycin per ml, and all but one Australian and one Malaysian strain were inhibited by 1 μg/ml (at pH 6.6).

Of the 46 strains tested at pH 6.6 and/or pH 7.4, 38 strains (83%) were inhibited by 0.5 μg/ml. For six Australian strains and one strain each from Benin and the Democratic Republic of Congo (formerly Zaire), the MICs were >0.5 μg/ml.

For some of the strains, the MICs obtained at pH 7.4 were 1 or more dilutions lower than those obtained at pH 6.6. MICs for 11 strains (7 from Australia, 2 from Benin, 1 from the Democratic Republic of Congo, and 1 from Togo) were ≤0.125 μg/ml when these strains were tested at pH 7.4. The MICs obtained on 7H11 agar (pH 6.6) for the Australian and Malaysian strains were higher by 1 to 2 dilutions than those for strains from other countries.

The MIC of clarithromycin for the ATCC reference strain, ATCC 19423, was 0.5 μg/ml on 7H11 medium (pH 6.6) and <0.125 μg/ml on Mueller-Hinton agar (pH 7.4).

DISCUSSION

The MICs for the 46 *M. ulcerans* strains tested at pH 6.6 and/or at pH 7.4 varied from ≤0.125 to 2 μg of clarithromycin per ml. For 1 Australian and 1 Malaysian strain (the latter tested at pH 6.6 only), the MIC was 2 μg/ml, while the other 44 strains were inhibited by concentrations lower than 2 μg/ml. These MICs are below peak plasma concentrations of clarithromycin obtainable in humans. Administration of 500 mg of clarithromycin twice daily for up to 3.5 days to volunteers resulted in mean peak plasma concentrations between 2.4 and 3.5 μg/ml (9).

For the majority of strains, geographic origin did not seem to play a significant role in sensitivity to clarithromycin. The Australian and the Malaysian strains, nevertheless, were more resistant; MICs for these strains ranged from 0.5 to 2 μg/ml. Genetic and phenotypic differences have been demonstrated between different strains of *M. ulcerans* (31). Whether these genetic and phenotypic differences (28) are related to susceptibility to antimycobacterial agents is unknown.

Low clarithromycin MICs have been obtained in vitro for other mycobacterial species; for example, with the BACTEC system, Rastogi and Goh (32) found low MICs for three *Mycobacterium bovis* BCG strains (0.25 to 0.5 μg/ml at pH 6.8 and 0.1 to 0.2 μg/ml at pH 7.4). These authors found MICs lower than 1 μg/ml at pH 6.8 and 7.4 for several other mycobacterial pathogens, including *Mycobacterium xenopi*, *Mycobacterium scrofulaceum*, *Mycobacterium kansasii*, *Mycobacterium chelonae*, and *M. marinum*. Using the broth microdilution method with Mueller-Hinton medium (pH 7.4), Brown et al. (7) found that 90% of *Mycobacterium chelonae* subsp. *chelonae* organisms and 90% of *Mycobacterium chelonae* subsp. *abscessus* organisms were inhibited by 0.25 and 0.5 μg of clarithromycin/ml, respectively. Rastogi and colleagues (33) also have reported clarithromycin activity against *M. marinum* and *Mycobacterium paratuberculosis* (MICs, 0.25 to 0.5 μg/ml). MICs at pH 7.4 in liquid media were 2 μg/ml or lower for *M. avium* strains (20). These results strongly suggest that clarithromycin is a promising chemotherapeutic agent for infections caused by a variety of mycobacterial species.

Because the inhibitory activity of clarithromycin is higher at pH 7.4 than at an acid pH, this drug may be especially effective for the elimination of extracellular bacteria and suppression of mycobacterial bacteremia. Heifets et al. (20) suggested that the elimination of *M. avium* from the blood of patients with AIDS under clarithromycin therapy was most likely related to the increased inhibitory activity of this drug at pH 7.4.

Clinical studies have repeatedly revealed that antimycobacterial agents are not effective for the treatment of advanced ulcerated lesions of BU. This is believed to be related to the low drug levels obtainable in the necrotic tissues of advanced BU in the skin. Extensive histopathologic studies demonstrate that *M. ulcerans* is essentially an extracellular parasite largely confined to areas of necrosis in skin; however, the bacillus often spreads to local and regional lymph nodes and causes metastatic disease in bone (1). In our report on 867 BU patients treated at Zangnanado (3), we observed disseminated lesions following the appearance of the primary lesion. In some instances the disseminated lesions appeared after complete

healing of the initial lesion. Such lesions probably represent hematogenous spread that may be preventable by effective antimycobacterial agents.

The present study demonstrates that levels of clarithromycin that are obtainable in blood are inhibitory for *M. ulcerans* in vitro. Thus, clarithromycin offers promise, not only for the treatment of early lesions of *M. ulcerans*, but also as an adjunct treatment for the prevention of hematogenous dissemination of the etiologic agent. The efficacy of rifampin against *M. ulcerans* in vitro and in vivo in the mouse has been established. The in vitro susceptibility of *M. ulcerans* to rifampin was found to be similar to that of *Mycobacterium tuberculosis* (18, 29, 34). Early nodular lesions and early limited ulcerated lesions in humans respond favorably to rifampin monotherapy (23). Rifampin, thus, could also be a useful drug for the prevention of dissemination.

Combined therapy is generally recommended for the treatment of mycobacterial infections in order to prevent the development of resistance when the infection is caused by a sufficiently large number of bacilli to allow for the selection of resistant mutants. Most BU patients, at some stage of the disease, have massive bacillary burdens. Multiple-drug therapy may also produce additive or mutually synergistic effects between the antibacterial agents. Although *M. ulcerans* is resistant in vitro to ethambutol (data not shown), several reports indicate that ethambutol is synergistic with other antimycobacterial agents (16, 33). Clarithromycin shows synergism with rifampin in combined therapy of *M. avium* complex infections, and ethambutol enhances the bactericidal activity of rifampin against the same *M. avium* strains (35, 39). It was, however, recently demonstrated that rifabutin in combination with clarithromycin substantially reduced clarithromycin levels (17). Although rifampin or clarithromycin alone may be used in the treatment of patients with BU, studies should be initiated to evaluate the effects of clarithromycin alone and in combination with ethambutol and rifampin on *M. ulcerans*, both in vitro and in vivo in experimentally infected mice. Such studies would give valuable information for the formulation of recommendations for an optimal antimycobacterial regimen for use (i) as a treatment of certain stages of *M. ulcerans* infection, (ii) for the prevention of dissemination during or after surgery, and (iii) as an adjunct to other therapeutic approaches. Secondary bacterial infections are common in BU patients, especially in patients with osteomyelitis (2). Multidrug regimens containing clarithromycin might help control the complications of BU, which are often mutilating and sometimes life threatening.

Finally, regimens containing rifampin and ethambutol would avoid the selection of resistant mutants in patients who may have undiagnosed *M. tuberculosis* infection.

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