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In vitro activity of ciprofloxacin, sparfloxacin, ofloxacin, amikacin and rifampicin against Ghanaian isolates of *Mycobacterium ulcerans*

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MICs of ciprofloxacin, sparfloxacin, ofloxacin, amikacin and rifampicin were determined for 14 primary clinical isolates and three reference isolates of *Mycobacterium ulcerans* by modifying a standard agar dilution method for testing *Mycobacterium tuberculosis* sensitivity. All these antimicrobials were active against every isolate of *M. ulcerans*. Sparfloxacin exhibited the highest activity and ofloxacin was the least effective. Rifampicin exhibited the broadest range of activity.

Introduction

Mycobacterium ulcerans infection causes skin ulcers called Buruli ulcer for which the mainstay of treatment is surgical excision followed by split thickness skin grafting. This is a significant health problem in West Africa, where its incidence is increasing, and it accounts for a large proportion of surgical bed occupancy.[1,2] In 1998 the WHO recognized Buruli ulcer as a major emerging disease and called for urgent action to control the disease.

M. ulcerans is resistant to some antituberculosis drugs *in vitro* including isoniazid and ethambutol,[3] and anecdotal evidence suggests that conventional antituberculosis drugs are not effective in treatment. The only clinical trial conducted so far demonstrated that clofazimine, an antileprosy agent, was not effective *in vivo* even though the organism is highly susceptible *in vitro*.[4] This may be due to poor perfusion of antibiotic into necrotic tissue where *M. ulcerans* proliferates in Buruli lesions. Nevertheless, antimicrobials could have a key role in the prevention of post-surgical recurrence of Buruli ulcer, which occurs frequently. The aim of this study was to determine the MIC of five antimicrobials including three fluoroquinolones against Ghanaian clinical isolates of *M. ulcerans* as a prelude to conducting clinical trials in Ghana.

Materials and methods

Bacterial isolates

Primary clinical isolates were obtained from lesions of patients from villages of the Amansie West district of the Ashanti region in Ghana. The samples were excised nodules of the pre-ulcerative stage of the disease, biopsies from the base of ulcers or swabs obtained from the undermined edges of ulcers. Tissue samples of approximately 1 mm^3 were ground manually using sterile sand and resuspended in saline. Decontamination of samples and primary isolation of *M. ulcerans* were performed as described previously.[5] Isolates were subcultured on to Middlebrook 7H11 oleic acid–albumin agar medium (Difco Laboratories, Detroit, MI, USA). To maintain isolates in continuous log phase, they were passaged on this medium every 4 weeks.

M. ulcerans reference isolate (TMC1615) was originally obtained from the Trudeau Mycobacterial Culture Collection, Saranac Lake, NY, USA. NCTC 10417 and NCTC 10445 were obtained from the NCTC collection, Public Health Laboratory Service, Colindale, London. All three isolates were originally from Buruli lesions. In total, 17 isolates were used for this study, including the reference isolates.

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Antimicrobial agents

The following antimicrobial agents were incorporated into 7H11 agar slopes: ciprofloxacin (Bayer PLC, Newbury, UK), sparfloxacin (Rhône DPC Europe, Antony Cedex, France), amikacin (NexStar Pharmaceuticals, San Dimas, CA, USA), ofloxacin and rifampicin (both from Sigma, Poole, UK). The concentrations used are shown in the Table.

Sensitivity testing

After initial standardization experiments, we modified the method of Canetti *et al.*[6] originally recommended for the testing of isoniazid resistance. A plastic inoculating loop (Fisher Scientific, Loughborough, UK) designed for sampling of 0.01 mL liquid volumes was used to transfer a loopful of culture (*c.* 5 mg wet wt) from the surface of a 7H11 slope to a 5 mL screw-capped bottle containing six glass beads and 0.2 mL of sterile water. The bottle was vortexed vigorously for 10 s and allowed to stand for 1 min; 0.8 mL of sterile water was then added, the suspension was mixed by shaking and the bottle was allowed to stand for 10 min after adding a fresh cap. After macroscopic clumps had settled to the bottom, a loopful of suspension (*c.* 0.01 mL) was taken from just under the surface of the liquid and placed on Middlebrook 7H11 oleic acid–albumin agar slopes with appropriate concentrations of antimicrobials and antimicrobial-free control slopes. Slopes were incubated at 31°C for 8 weeks. Initial readings were taken after the fourth week and definitive readings were taken when there were between 1000 and 2000 colonies on control slopes. Occasionally, testing had to be repeated when colony numbers were outside this range. The lowest concentration of antimicrobial that completely inhibited growth (zero colonies) was taken as the MIC.

Table. MICs of antimicrobials tested against 14 Ghanaian clinical isolates and three reference isolates

	Concentration of antimicrobial (mg/L)				
	0.1[a]	0.25	0.5	1	2
Rifampicin	2	8	12	16	17
Amikacin		1	15	17	
Ofloxacin				2	17
Ciprofloxacin		8	16	17	
Sparfloxacin	1	16	17		

Cumulative number of isolates of *M. ulcerans* ($n = 17$) exhibiting complete inhibition of growth.

[a]This was the lowest concentration of antimicrobial used for all sensitivity testing, hence the actual MIC for isolates inhibited by this concentration may be lower.

Results

The Table summarizes the results. Sparfloxacin exhibited the highest antimycobacterial activity and the majority of isolates were inhibited by 0.25 mg/L. All isolates were inhibited by both amikacin and ciprofloxacin at 1 mg/L. Ofloxacin appeared to be the least effective, but all isolates were inhibited at concentrations of 2 mg/L. The broadest range of sensitivity was to rifampicin.

Discussion

In all but three Ghanaian isolates, sensitivity could be determined after 4 weeks of incubation. For the remaining three isolates, results were obtained 7–8 weeks after inoculation because they had a slower growth rate. Sharp end-points were obtained for all isolates, and MICs for slower and faster growing isolates were comparable. No pseudo-resistance was observed in the faster growing isolates during weeks 5–8, reflecting the highly bactericidal nature of the antimicrobials used. MICs obtained for all isolates were thus considered to be reliable despite variations in growth rate.

Our study demonstrates that *M. ulcerans* is highly susceptible to ciprofloxacin, ofloxacin, sparfloxacin, amikacin and rifampicin, and to our knowledge there are no previous reports of susceptibility to quinolones. Quinolones are known to penetrate well into tissues, in addition to having strong antimycobacterial activity.[7] Susceptibility to rifampicin has been reported previously,[8] thus validating our testing method. More recently, clarithromycin has also been found to be highly effective against a variety of isolates, including Ghanaian isolates.[9]

As primary antibiotic therapy has been found to be ineffective, lesions are treated by surgical excision followed by skin grafting if required. Local recurrence is as high as 16%.[10] This is because the margins of the lesion are sometimes difficult to define and residual organisms continue to proliferate. The aim of surgery is to remove visibly necrotic tissue and a margin of normal tissue. Any remaining infected tissue is likely to be penetrable by antibiotics, unlike the necrotic central part of the lesion. Therefore antimicrobials may have a role in prevention of recurrences, but this has yet to be established. In a study in Benin, disseminated forms of infection were observed in 10% of cases, and in some instances these occurred after post-surgical healing of the initial lesion,[2] probably as a result of haematogenous spread. This may be another indication for prophylactic antimicrobials. The results of our study together with evidence from other *in vitro* and *in vivo* studies pave the way for clinical trials aimed at reducing the frequency of recurrence.

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