In vitro activity of sitafloxacin (DU-6859a) alone, or in combination with rifampicin, against Mycobacterium ulcerans

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The antimicrobial effect of sitafloxacin (DU-6859a), used either singly or in combination with rifampicin, was evaluated in vitro against Mycobacterium ulcerans. Growth of M. ulcerans was measured by plate counts and the BACTEC radiometric method. The MICs and MBCs of sitafloxacin for M. ulcerans were in the range 0.125–0.5 mg/L. The values for other fluoroquinolones were two- to four-fold higher than for sitafloxacin. Combination of sitafloxacin and rifampicin exhibited synergy with five of the eight strains, whereas the combination of ofloxacin and rifampicin resulted in additive effects only. These results suggest that the combination of sitafloxacin and rifampicin has potential in the treatment of M. ulcerans infection.

Keywords: sitafloxacin, Mycobacterium ulcerans, ofloxacin, rifampicin

Introduction

Infection with Mycobacterium ulcerans causes a deforming disease that clusters in swampy lowlands and river valleys in tropical regions. Although the organism was first documented in Bairnsdale, South Australia in 1947,1 the disease was named after the Buruli District of Uganda after an investigation of superficial, ulcerating lesions in Ugandan children.2 In recent years, Buruli ulcer disease has assumed public health importance in many countries, prompting the establishment of the Global Buruli Ulcer Initiative by the World Health Organization in early 1998.3 The lesions often start as small subcutaneous nodules, gradually enlarge over days to weeks and lead to painless and chronic ulcers with characteristic undermined edges.

Despite the promising results in vitro4 and in laboratory animals,5 the treatment of these ulcers has been disappointing, especially in patients with extensive ulcers; large surgical excision of the necrotic tissue followed by skin grafting is, at present, the only treatment. Even though M. ulcerans is resistant to many antituberculosis drugs in vitro, antimicrobials could play a key role in the prevention of post-surgical recurrence of Buruli ulcers, which occurs frequently.

A new fluoroquinolone, sitafloxacin (DU-6859a), has been shown to be more potent than ofloxacin against mycobacteria, including M. tuberculosis.6 Thus, the aim of this study was to determine the activity of sitafloxacin, along with standard quinolones, used singly or in combination with rifampicin, against M. ulcerans.

Materials and methods

Organisms

Two type strains of M. ulcerans were used: ATCC 19423 and ATCC 35840. Six other M. ulcerans strains isolated from six countries were also included in this study. Isolates and type strains were maintained on Lowenstein–Jensen medium. Whenever needed, colonies from Lowenstein–Jensen medium were subcultured in Middlebrook 7H9 broth containing OADC enrichment (where OADC stands for oleic acid + albumin + dextrose + catalase).

Antimicrobial agents

Sitafloxacin, (–)-7[(7S)-amino-5-azaspiro{2,4}heptan-5-yl]-8-chloro-6-fluoro[(1R,2S)-2-fluoro-1-cyclopropyl]-1,4-dihydro-4-oxo-3-quinolincarboxylic acid sesquihydrate, also
known as DU-6859a, was obtained from Daiichi Pharmaceutical Company, Tokyo, Japan. Stock solutions of sitafloxacin, and levofloxacin and ofloxacin (both from R. W. Johnson Pharmaceutical Research Institute, Ranitan, NJ, USA) were prepared fresh in 0.1 M NaOH. Stock solution of ciprofloxacin (Miles Laboratories, West Haven, CT, USA) was prepared in distilled water. Stock solution of rifampicin (Sigma Chemical Company, St Louis, MO, USA) was prepared by dissolving first in small volumes of methanol and diluting further in water. Stock solutions were further diluted appropriately in distilled water to prepare working solutions that were filter sterilized through a GA-6 membrane filter (pore size 0.22 µm; Gelman Sciences, Ann Arbor, MI, USA).

The procedures described by Heifets et al.² (BACTEC method and plate counts) were carried out to determine the MICs and MBCs, and also to assess the combined inhibitory effects. To assess the combined inhibitory effects, each drug was tested at concentrations below its MIC in serial two-fold dilutions (e.g. 2+2, 2+4 and 2+8, etc.), and the interaction indicator or fractional inhibitory concentration (FIC) was calculated. For each sample, control as well as with drug, triplicate assays were carried out in each case and statistical significance was determined by the Student’s t-test.

Results

The effects of sitafloxacin, along with other fluoroquinolones and rifampicin, incorporated singly into 7H12 medium, against M. ulcerans are presented in Table 1. The effect of an individual drug was evaluated by determining the inhibition of M. ulcerans caused by that drug as compared with the growth of M. ulcerans in control cultures without any drug. The results presented in Table 1 are highly significant, with P values ≤0.05. Among the fluoroquinolones, sitafloxacin was the most potent in killing M. ulcerans. The MIC and MBC values of sitafloxacin for eight strains varied between 0.125 and 0.5 mg/L; but more importantly, in six of eight strains, including four clinical isolates, the MBC/MIC ratio was 1.0. In the case of ciprofloxacin, levofloxacin and ofloxacin, MIC and MBC values ranged between 0.25 and 4.0 mg/L, with seven of eight strains (in the case of levofloxacin, all eight strains) showing MBC/MIC ratios of 2 and the one remaining strain showing a ratio of 4.

The results obtained when either sitafloxacin or ofloxacin was combined with rifampicin, with each drug at concentrations lower than their respective MICs, are presented in Table 2. The combination of sitafloxacin and rifampicin exhibited synergy in five of the eight strains of M. ulcerans, with an FIC value of 0.5. In the case of two strains, the FIC value was 0.75, whereas in one strain an additive effect was seen, with an FIC value of 1.0. On the other hand, additive effects were observed with all eight strains of M. ulcerans tested with a combination of ofloxacin and rifampicin with FIC values of 1.0 for all the strains.

Discussion

The data presented here clearly demonstrate the superiority of sitafloxacin (DU-6859a) over other fluoroquinolones, including ofloxacin, in inhibiting the in vitro growth of M. ulcerans. Such superiority of sitafloxacin also has been demonstrated in the case of in vitro as well as in vivo growth of Mycobacterium leprae (A. M. Dhople and K. Namba, unpublished results). In the present study, the fact that synergy was exhibited with the combination of sitafloxacin and rifampicin, but not with a combination of ofloxacin and rifampicin, is noteworthy. These observations have further been confirmed by the current authors in in vivo studies using the mouse footpad system (A. M. Dhople and K. Namba, unpublished results).

The non-AIDS-associated infections caused by non-tuberculosis mycobacteria are increasing. Among these, infection caused by M. ulcerans poses the greatest public

Table 1. In vitro susceptibilities of M. ulcerans to sitafloxacin, other fluoroquinolones and rifampicin, used singly

<table>
<thead>
<tr>
<th>Strain/isolate</th>
<th>MIC (mg/L)</th>
<th>MBC (mg/L)</th>
<th>MBC/MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SIT</td>
<td>OFX</td>
<td>LVX</td>
</tr>
<tr>
<td>ATCC 19423</td>
<td>0.125</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>ATCC 35840</td>
<td>0.125</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>94-886</td>
<td>0.125</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>94-539</td>
<td>0.25</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>5143</td>
<td>0.125</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>842</td>
<td>0.125</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>8756</td>
<td>0.125</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>5155</td>
<td>0.25</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

SIT, sitafloxacin; OFX, ofloxacin; LVX, levofloxacin; CIP, ciprofloxacin; RIF, rifampicin.
health threat and thus is rapidly becoming the third most prevalent mycobacterial disease after tuberculosis and leprosy. Among the various antimycobacterial drugs that have already been tested in vitro against M. ulcerans, only rifampicin was evaluated in mice and found to be effective. However, pre-ulcerative and early ulcerative, but not advanced ulcers, can be effectively treated with rifampicin. Recently, Thangaraj et al. have shown superiority of sparfloxacin over ofloxacin and ciprofloxacin in inhibiting the in vitro growth of M. ulcerans. However, sparfloxacin was later found to be ineffective against M. ulcerans infection in mice. Most Buruli ulcer patients carry massive bacterial loads at some stage of the disease, thus creating an ideal situation for the selection of drug-resistant mutants. In such a situation, combined therapy, as in any other mycobacterial infection, will be highly advantageous. Since combination of sitafloxacin and rifampicin is effective in both in vitro as well as in vivo studies, such a combination could have potential for the chemotherapeutic treatment of advanced ulcers.

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References


