Postantibiotic Effects of Amikacin and Ofloxacin on *Mycobacterium fortuitum*  

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A study of postantibiotic effects (PAE) in vitro of amikacin and ofloxacin on *Mycobacterium fortuitum* isolates from sternotomy wounds by use of the dilution method for drug removal showed that both drugs exhibited good bactericidal activities, with the PAE of amikacin lasting from 13.5 to 27.6 h and the PAE of ofloxacin lasting from 1.2 to 5.0 h. These laboratory results concur with our experience of the efficacy of once-daily dosing with these drugs in the treatment of infections caused by *M. fortuitum*. These data may have therapeutic implications in guiding the scheduling of the administration of drugs in these infections, which require a long duration of therapy.

Postantibiotic effects (PAE) refer to the continuation of the suppression of the growth of microorganisms following limited periods of exposure (1, 8, 14). Amikacin and ofloxacin have been shown to have good in vitro activities against *Mycobacterium fortuitum* (2, 11, 12, 15, 18, 21), and the therapeutic outcome of patients with infections caused by this pathogen and treated with these drugs is also favorable (16–20). Since these rapidly growing mycobacteria have mean generation times only severalfold that of common bacteria, we thought that it would be pertinent to study the PAE of amikacin and ofloxacin on *M. fortuitum*. Earlier studies concerning pulsed exposure of slowly growing *M. tuberculosis* to conventional antituberculous agents for 6 to 96 h have allowed therapeutic hints to be derived concerning the chemotherapy of tuberculosis, in particular the utility of intermittent regimens (3, 4, 9). By analogy, these PAE studies of *M. fortuitum* may also help to guide the formulation of chemotherapeutic regimens for *M. fortuitum* infections.

MATERIALS AND METHODS

Bacterial strains and their maintenance. Working cultures of a standard strain of *M. fortuitum* (ATCC 6841) and five different clinical isolates derived from sternotomy wound infection sites were made on Lowenstein-Jensen medium and on Mueller-Hinton agar (Oxoid) incubated aerobically at 37°C. Stock cultures were maintained on Lowenstein-Jensen medium at room temperature and at 4°C in and Middlebrook 7H9 broth (Difco) in liquid nitrogen at −180°C.

MIC and MBC determinations. In vitro susceptibilities to amikacin (Bristol-Meyers Pharmaceuticals) and ofloxacin (Daiichi Pharmaceuticals) were determined by the broth dilution method. Standard drug powders were dissolved in sterile distilled water to yield stock solutions with a concentration of 6.4 mg/ml. Serial twofold dilutions were then made in Mueller-Hinton broth to yield drug concentrations in the range of 2.0 to 64.0 mg/liter for amikacin and 0.5 to 8.0 mg/liter for ofloxacin.

Working cultures of the *M. fortuitum* isolates in Mueller-Hinton broth were incubated at 37°C for 2 to 3 days. The cultures were then diluted with sterile distilled water to match the turbidity of a McFarland no. 1 standard, and 0.1 ml of each culture was used as the inoculum for each 10 ml of drug-containing medium. The inoculum size was 10^7 to 10^8 CFU/ml, as determined by the viable count method. *Escherichia coli* NCTC 10418, *Pseudomonas aeruginosa* ATCC 37919, and *Staphylococcus aureus* NCTC 6571 were used as controls.

After inoculation, the culture bottles were incubated aerobically at 37°C. The MICs were defined as the lowest concentrations that inhibited macroscopically visible growth after 3 days of incubation. The MBCs for all the isolates were determined by subculturing onto Mueller-Hinton agar at the end of 3 days of incubation. All experiments were performed in duplicate.

Experimental design for the study of PAE. The *M. fortuitum* isolates were subcultured in Mueller-Hinton broth until the optical density of each was about 10 times that of a McFarland no. 1 standard. A 0.5-ml quantity of each subculture was mixed with 4.5 ml of Mueller-Hinton broth and incubated at 37°C for 16 h so that the bacteria would reach the logarithmic phase of growth. Each final culture suspension was found to contain about 10^8 CFU/ml by the viable count method. For each of these, the following procedure was carried out. One milliliter of cell suspension was added to 9.0 ml of warm Mueller-Hinton broth and designated the control drug-free tube for study of the growth curve for *M. fortuitum*. Another 1.0 ml of the same suspension was added to 9.0 ml of warm Mueller-Hinton broth containing either appropriate antimicrobial agent (amikacin, 32 mg/liter; ofloxacin, 8.0 mg/liter). After 2 h of incubation at 37°C, a 0.1-ml aliquot was pipetted out of the control tube and 99.9 ml of warm Mueller-Hinton broth was added to this tube to achieve dilution up to 1,000-fold. Similarly, for a 0.1-ml aliquot of each of the aforementioned antimicrobial agent-containing suspensions, the antimicrobial agent was removed by 1,000-fold dilution of the suspension with warm drug-free Mueller-Hinton broth for later PAE studies. Furthermore, a 0.1-ml aliquot of each of the antimicrobial agent-containing suspensions was similarly diluted with warm Mueller-Hinton broth containing the corresponding antimicrobial agent (amikacin, 32 mg/liter; ofloxacin, 8.0 mg/liter) for the study of bactericidal effects. After all of

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TABLE 1. MICs, MBCs, PAE, and KIs of amikacin and ofloxacin for M. fortuitum

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>MIC (MBC), mg/liter, of:</th>
<th>PAE (KI), h, of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amikacin</td>
<td>Ofloxacin</td>
</tr>
<tr>
<td>ATCC 6841</td>
<td>8 (8)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Clinical isolates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8 (8)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>2</td>
<td>4 (4)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>3</td>
<td>≤2 (4)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>4</td>
<td>≤2 (≤2)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>5</td>
<td>≤2 (≤2)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

these procedures were done, incubation was continued at 37°C. Aliquots of 0.1 ml of the suspensions were obtained at regular intervals of 4 to 5 h until a maximum of 60 h had elapsed. Serial 10-fold dilutions of these aliquots up to 10⁻⁸ were made for viable count studies with Mueller-Hinton agar. Only dilutions that yielded 30 to 150 CFU on the plates were recorded, and viable counts were calculated therefrom. This criterion was not observed for bactericidal-effect studies because the counts were much lower. All experiments were performed in duplicate.

Calculation of mean generation times, in vitro PAE, and KIs. The mean generation time of an organism is defined as the time required for it to increase in number by 1 log₁₀ CFU per milliliter on the control curve generated by the viable count method. The in vitro PAE were calculated as previously described with the formula PAE = T − C, where T is the time required for the CFU per milliliter in the test culture to increase by 1 log₁₀ unit above the value immediately after drug removal and C is the time required for the CFU per milliliter in the drug-free control culture to increase by 1 log₁₀ unit immediately after the same dilution procedure to mimic drug removal (1, 8, 14). The killing index (KI) is defined as the time required for a decrease in the CFU per milliliter of 1 log₁₀ unit on the bactericidal effect curve on continuous exposure to the respective antimicrobial agents.

RESULTS

The MICs of amikacin against the M. fortuitum isolates ranged from ≤2.0 to 8.0 mg/liter; the range of MBCs was similar (Table 1). The MICs of ofloxacin against the same isolates ranged from 1.0 to 4.0 mg/liter, and the MBCs ranged from 1.0 to 8.0 mg/liter (Table 1). The mean generation times of the M. fortuitum isolates studied were 3.1 ± 0.5, 2.9 ± 0.6, and 3.1 ± 0.5 h, as revealed by the control growth curve and the PAE studies with amikacin and ofloxacin, respectively (Fig. 1 and 2). PAE were observed for all M. fortuitum isolates tested against amikacin (Fig. 1) and ofloxacin (Fig. 2), with that of amikacin lasting from 13.5 to 27.6 h (Table 1) and that of ofloxacin lasting from 1.2 to 5.0 h (Table 1). Bactericidal effects of these two drugs on M. fortuitum isolates were also observed (Fig. 1 and 2), with KIs ranging from 2.4 to 5.9 h for amikacin and 2.6 to 4.6 h for ofloxacin (Table 1). No regrowth was observed after prolonged incubation of the isolates in the antimicrobial agent-containing media.

DISCUSSION

Our experience with once-daily or twice-daily dosing of amikacin in combination with ofloxacin for the treatment of 10 patients with M. fortuitum sternotomy wound infections was rather rewarding (16). We have also been able to use high doses of ofloxacin administered once daily very effectively for the treatment of M. fortuitum sternotomy wound and lung infections (16, 17, 19, 20). These infections, being basically difficult to treat, might entail months of therapy, a duration that involves problems with patient compliance and drug toxicity (7). Knowledge of the PAE of these antimicrobial agents on M. fortuitum might help to provide further guidance in therapy for combating these two problems logistically.

When tested in vitro, PAE depend on a number of factors, including the type of organism, class of antimicrobial agent, drug concentration used, and duration of exposure (14). We chose the usual therapeutic levels of amikacin and ofloxacin in serum for PAE testing because such conditions would closely resemble the in vivo setting. A duration of exposure of 2 h was used on the basis of experience reported in the literature for more rapidly growing bacteria (10, 14). We found that the mean generation time of M. fortuitum was not affected by preexposure to the antimicrobial agents.

Our results demonstrated distinct in vitro PAE of amikacin on M. fortuitum lasting as long as 13.5 to 27.6 h (Table 1 and Fig. 1). We also found that PAE were more pronounced.

FIG. 1. PAE (+) and bactericidal effect (•) of amikacin on M. fortuitum ATCC 6841. The time between the arrowheads denotes the time during which PAE occurred. ■, control.
for isolates for which MICs were lower. This result may be a corollary of the positive correlation between administered drug concentration and PAE in vitro (14). On the basis of this laboratory finding, a once-daily dosing regimen would be justified, despite the fact that the elimination half-life of amikacin given parenterally is only 2 to 3 h (6). Our observation of the in vitro PAE of ofloxacin lasting 1.2 to 5.0 h (Table 1 and Fig. 2) does not favor completely the use of a single-daily dosing regimen when PAE alone are considered in designing the regimen. The clinical success of a single-daily dosing regimen for ofloxacin may be due to the long half-life (5 to 6 h) and effective penetration of this antimicrobial agent into tissues (5, 13). We conclude that the design of a dosing regimen for treating *M. fortuitum* infections depends on several factors, including the pharmacokinetics of the antimicrobial agents, susceptibilities in vitro, and PAE. Confirmation of the efficacy of such a regimen can be furnished only by clinical success.

ACKNOWLEDGMENTS

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REFERENCES


