Postantibiotic Effects and Bactericidal Activities of Levofloxacin and Gatifloxacin at Concentrations Simulating Those of Topical Ophthalmic Administration against Fluoroquinolone-Resistant and Fluoroquinolone-Sensitive Methicillin-Resistant \textit{Staphylococcus aureus} Strains

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Postantibiotic Effects and Bactericidal Activities of Levofloxacin and Gatifloxacin at Concentrations Simulating Those of Topical Ophthalmic Administration against Fluoroquinolone-Resistant and Fluoroquinolone-Sensitive Methicillin-Resistant Staphylococcus aureus Strains

Saichi Hoshi,1,2 Ken Kikuchi,1* Takashi Sasaki,1 Chie Sotozono,2 Shigeru Kinoshita,2 and Keiichi Hiramatsu1

Department of Infection Control Science, Faculty of Medicine, Juntendo University, Tokyo 113-8421, Japan,1 and Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto 602-0841, Japan2

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The bactericidal activities and postantibiotic effects (PAEs) of levofloxacin and gatifloxacin at concentrations corresponding to those in antibiotic eye drops against methicillin-resistant Staphylococcus aureus strains were determined. Levofloxacin and gatifloxacin at concentrations simulating those in eye drops showed lower bactericidal activities and shorter PAEs against fluoroquinolone-resistant strains than against fluoroquinolone-sensitive strains.

Ocular infections caused by methicillin-resistant Staphylococcus aureus (MRSA) strains, which are clinically serious and difficult to treat, have been reported (18, 27–30, 34). The prevalence of levofloxacin (LVX) resistance in MRSA strains isolated from patients with ocular infections is 80 to 90% in Japan and the United States (12, 14, 17). Topical treatments may have selected for strains with high-level fluoroquinolone resistance (1, 8, 10, 14, 17), which is associated with multiple mutations in the quinolone resistance-determining regions (QRDRs) of MRSA strains isolated at ophthalmologic clinics (10). Although topical fluoroquinolone eye drops contain much higher concentrations (0.3 to 1.5%; equivalent to 3,000 to 15,000 μg/ml) than their MICs even against strains with high-level fluoroquinolone resistance, the antibiotic concentrations in tear films decline rapidly (by about 1/100 at 30 min) (31), and treatment failures have been noted (6, 33). We performed time-kill and postantibiotic effect (PAE) studies with LVX and gatifloxacin (GAT) at concentrations simulating those of topical ophthalmic administration against fluoroquinolone-resistant and -sensitive MRSA strains to examine the

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (μg/ml)</th>
<th>Type of LVX resistance</th>
<th>Amino acid or nucleotide change in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OXA</td>
<td>LVX</td>
<td>LVX + reserpine</td>
</tr>
<tr>
<td>JCSC 6763</td>
<td>512</td>
<td>512</td>
<td>512</td>
</tr>
<tr>
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<td>JCSC 6766</td>
<td>512</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
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<td>256</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>JCSC 6768</td>
<td>256</td>
<td>4</td>
<td>2</td>
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<td>JCSC 6769</td>
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<tr>
<td>JCSC 6770</td>
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<td>0.25</td>
</tr>
<tr>
<td>JCSC 6822</td>
<td>128</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>

a No changes were detected in grfB.

b Portion of the norA gene from nucleotides 305 to 476 encompassing the −35 and −10 sequences in the promoter region representing the transcriptional start site described by Yoshida et al. (35)

* Corresponding author. Mailing address: Department of Infection Control Science, Faculty of Medicine, Juntendo University, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan. Phone: 81-3-3813-3111, ext. 3822. Fax: 81-3-3816-2782. E-mail: kikuti@med.juntendo.ac.jp.

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efficacies of these drugs. We examined nine typical Japanese health care facility-associated MRSA strains: staphylococcal cassette chromosome mec type IIa (3, 13, 22, 24) strains with high-level LVX resistance (LHR; MICs, $\geq 64 \mu g/ml$), low-level LVX resistance (LLR; MICs, 4 to 32 $\mu g/ml$), or LVX sensitivity (LS; MICs, $\leq 1 \mu g/ml$). Cation-adjusted Muller-Hinton II broth (CAMHB; BD, Sparks, MD) and Mueller-Hinton agar (BD) were used for determination of the MICs, time-kill profile analyses, PAE assays, and viable count assays. MICs were measured by broth microdilution methods and were interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (4). To elucidate the contribution of efflux mediated by NorA to fluoroquinolone resistance, the MICs of LVX and GAT were determined in the presence or absence of 20 $\mu g/ml$ reserpine, which is a multidrug transport inhibitor (21). The QRDRs and norA promoter regions were sequenced as reported previously (10, 20, 21, 35). Time-kill assays were performed according to previously published...
Bactericidal activity was defined as a 24 h. Serial 10-fold dilutions in sterile saline were plated, and were taken at 0 min, 5 min, and 30 min and at 1, 2, 4, 6, and 1 standard deviation (bars). MRSA strains after 5 min of exposure to the antibiotics. Each test group comprised three different strains. The data are presented as means with 1 standard deviation (bars). LVX and GAT were used at 4/106 CFU/ml LVX or 2,000 g/ml GAT for 5 min; these antibiotic concentrations were derived from the areas under the concentration-time curves of LVX and GAT in tears after the administration of a single eye drop (25, 31). After removal of the drugs and six washes with saline, the membrane-trapped bacteria were resuspended in prewarmed CAMHB and reincubated (5). Control bacteria not exposed to drug were washed completely by LVX and GAT at 4/106,8/106, and 16/106 CFU/ml. Samples close to that present in eye drops, LVX completely killed two of three LHR strains, two of three LLR strains, and two of three LS strains, whereas GAT completely killed two of three LHR strains, two of three LLR strains, and three of three LS strains at 60 min. All the strains were killed completely by LVX and GAT at 4×, 8×, and 16× the MICs and at 2,048 μg/ml after 24 h of exposure (data not shown). Overall, GAT was more bactericidal than LVX for the MRSA strains. Similar findings have been obtained in previous studies, and it has been suggested that the differences observed are attributable to the C-8 methoxy moiety (2, 11). The PAEs of LVX and GAT were presented in Fig. 3. The PAEs of both drugs against the LHR and LLR strains were shorter in duration than those against the LS strains (Fig. 3A). There was a linear correlation between the PAEs of both drugs and their MICs (Fig. 3B). Since LVX and GAT at sub-MICs show PAEs against S. aureus strains (15, 23), the infrequent ophthalmic administration of both drugs may be effective against ocular infections caused by LS strains. The shorter PAEs and lower bactericidal activities against LHR and LLR strains compared to those against LS strains may affect clinical outcomes. The maintenance of adequate concentrations of fluoroquinolones (preferably GAT) by frequent administration may be more clinically efficacious against fluoroquinolone-resistant strains. Indeed, the frequent administration of eye drops containing GAT has successfully been used to treat moderately or highly resistant S. aureus strains (MICs, 12 μg/ml and >64 μg/ml, respectively) in a rabbit keratitis model (26). However, high concentrations of fluoroquinolones can cause corneal perforations in humans (7, 16, 32). We did not investigate how the growth-inhibitory effects of long-duration supra-MIC and sub-MIC dosages of antibiotics following brief exposure to a high concentration of the antibiotic affected the antibacterial responses. Further studies are required to determine the usefulness of these two antibiotics in topical formulations (eye drops) in different in vitro simulation models that reproduce the pharmacokinetic-pharmacodynamic properties of the antibiotics in the human eye when they are administered on traditional dosing schedules.

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