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Uptake of Sparfloxacin and Norfloxacin by Clinical Isolates of *Staphylococcus aureus*

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The amount of sparfloxacin uptake was higher than that of norfloxacin uptake in *Staphylococcus aureus*. Moreover, energy-dependent reduction in quinolone uptake, probably due to active efflux of the quinolone, was observed. The reduction in quinolone uptake appeared to be associated with quinolone resistance in *S. aureus*.

The fluoroquinolones have potent activity against gram-positive and gram-negative bacteria. Recently, a fluoroquinolone, sparfloxacin (formerly AT-4140), was found to have greater activity against clinical isolates of *Staphylococcus aureus*, including some quinolone-resistant *S. aureus* isolates, than were the existing quinolones (12, 13). In gram-negative rods such as *Escherichia coli*, the mechanisms of quinolone resistance were attributed to a decrease in outer membrane permeability (2, 5, 7–9) and an alteration in DNA gyrase (1, 8, 9, 11, 14, 15). With respect to quinolone permeation, active efflux of norfloxacin in *E. coli* has been suggested (5). However, little is yet known about the mechanisms of quinolone resistance or quinolone permeation in gram-positive cocci such as *S. aureus*. This report describes the uptake of sparfloxacin and norfloxacin by *S. aureus* with regard to quinolone resistance.

A total of 212 clinical isolates of *S. aureus* collected in Japan were divided into three types on the basis of their susceptibilities to sparfloxacin and norfloxacin (12). Isolates of the first type were susceptible to sparfloxacin and norfloxacin. The second type was susceptible to sparfloxacin (MIC, ≤0.39 μg/ml) but resistant to norfloxacin. The third type was resistant to both quinolones. For each type, typical isolates, the origins of which were independent of each other, were selected for the present study. *S. aureus* MS16008, of the first type, was isolated from sputum in 1985. *S. aureus* MS16401, of the second type, was isolated from pus in 1987. *S. aureus* NMS54, of the third type, was isolated from milk from a patient with mastitis in 1988. Sparfloxacin (12) was obtained from Dainippon Pharmaceutical Co., Ltd., Osaka, Japan, and norfloxacin was obtained from Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan. MICs were determined by the twofold agar dilution method with a final inoculum size of approximately 5×10^3 CFU per spot after 18 h of incubation at 37°C (12). The uptake of sparfloxacin and norfloxacin by the above-described isolates was examined by the method of Hirai et al. (7). The quinolone was added to the bacterial culture to a final concentration of 10 μg/ml unless otherwise indicated. When needed, an energy inhibitor, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP; Sigma Chemical Co., St. Louis, Mo.), was added to the culture to a final concentration of 100 μM 10 min after the addition of the quinolone (3, 4, 10). The amounts of cell-associated quinolones were measured by high performance liquid chromatography (Irica Instruments Inc., Kyoto, Japan) with a YMC A-312 column (Yamamura Chemical Laboratories Co., Ltd., Kyoto, Japan). The mobile phases were 5% acetic acid–methanol–acetonitrile (77:14:9 [vol/vol/vol]) for sparfloxacin and 5% acetic acid–methanol (85:15 [vol/vol]) for norfloxacin. The wavelengths of detection were 300 nm for sparfloxacin and 278 nm for norfloxacin.

The susceptibilities of the *S. aureus* isolates used in this study to sparfloxacin and norfloxacin are shown in Table 1. The amount of quinolone uptake by *S. aureus* MS16008, when tested 10 min after the addition of the drug, increased linearly with an increase in the extracellular quinolone concentration (Fig. 1). This linear relationship was seen over a range of extracellular concentrations from 0.5 to 80 μg/ml for sparfloxacin and 2.5 to 40 μg/ml for norfloxacin. In the case of norfloxacin, the amount of uptake at an extracellular concentration of 80 μg/ml was approximately 50% greater than that expected on the basis of the line obtained with concentrations of 2.5 to 40 μg/ml. These results indicated that the uptake of both quinolones by MS16008 was not saturable up to an extracellular concentration of 80 μg/ml. The binding of both quinolones to MS16008 seemed to be reversible, because approximately 90% of cell-associated sparfloxacin and approximately 80% of cell-associated norfloxacin, respectively, were removed by two additional washings of the cells with warm saline. The uptake of both quinolones by MS16008 reached plateau levels within 5 min, and MS16008 took up higher amounts of both quinolones than did any of the other isolates tested (Fig. 2A). The level of sparfloxacin uptake was approximately six-fold higher than was that of norfloxacin uptake in MS16008. *S. aureus* MS16401 showed less uptake of norfloxacin than did *S. aureus* MS16008, but sparfloxacin uptake by MS16401 remained at a relatively high level (Fig. 2B). *S. aureus* MS16658, which was isolated from sputum in 1989, resembled MS16401 in its profile of susceptibility to sparfloxacin and norfloxacin. The MICs of sparfloxacin and norfloxacin for MS16658 were 0.1 and 50 μg/ml, respectively, and the uptake of both quinolones was comparable to that by MS16401 (data not shown). Presumably, a reduced uptake of norfloxacin was correlated with norfloxacin resistance in MS16401 and MS16658. In *S. aureus* NMS54, sparfloxacin uptake was also reduced as compared with that in MS16008, MS16401, and MS16658 (Fig. 2C). NMS54 was highly resistant to both sparfloxacin and norfloxacin, and the reduction of sparfloxacin uptake may have been associated with sparfloxacin resistance.

On the other hand, the uptake of both quinolones by the isolates tested increased rapidly after the addition of CCCP

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TABLE 1. Susceptibilities of *S. aureus* isolates to sparfloxacin and norfloxacin

Strain	MIC (μg/ml)	
	Sparfloxacin	Norfloxacin
MS16008	0.025	0.78
MS16401	0.2	200
NMS54	50	>400

(Fig. 2A, B, and C). CCCP blocks energy production by destroying proton motive force (5). The higher level of quinolone uptake in CCCP-treated, deenergized cells than in non-CCCP-treated, energized cells suggested either the presence of active efflux of the quinolone in *S. aureus*, as previously described for *E. coli* (5, 6, 10) and *Pseudomonas aeruginosa* (3, 4), or active reduction of quinolone influx. The susceptible *S. aureus* cells took up more quinolone than did the resistant *S. aureus* cells in the absence of CCCP, but this difference in quinolone uptake was abolished by CCCP. This result suggested that the energy-dependent process played an important role in reducing quinolone uptake in quinolone-resistant isolates. Although an alteration in the DNA gyrase of these isolates was not examined, our results indicated that a reduced uptake of a quinolone may affect the susceptibility of *S. aureus* to the quinolone.

There was a difference in antibacterial activity and uptake between sparfloxacin and norfloxacin, as seen typically in MS16401. The levels of sparfloxacin uptake were higher than were those of norfloxacin regardless of the absence or the presence of CCCP. The results suggested improved penetrability of sparfloxacin into the *S. aureus* cells and, if active efflux occurs, more efficient efflux of norfloxacin than of sparfloxacin. We infer that the characteristics of sparfloxacin permeation may be related to the moderate lipophilicity of the sparfloxacin molecule (data not shown).

Further examinations of quinolone uptake and studies of the inhibitory effects of quinolones on DNA gyrase will be important in elucidating the mechanisms of quinolone resistance in gram-positive cocci, including *S. aureus*.

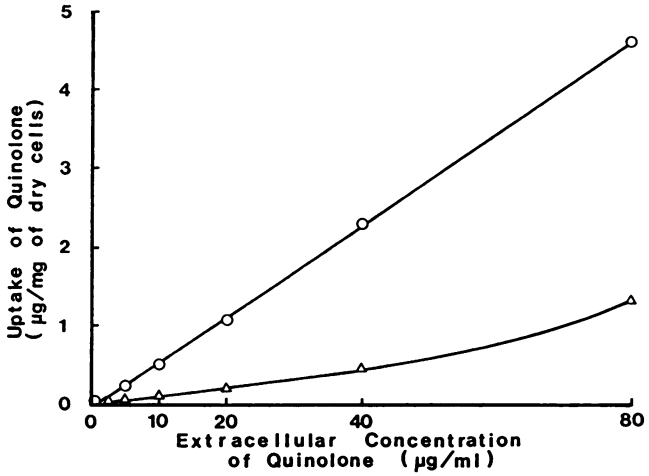

FIG. 1. Relationship between extracellular quinolone concentration and amount of quinolone uptake in *S. aureus* MS16008. Symbols: ○, sparfloxacin; △, norfloxacin.

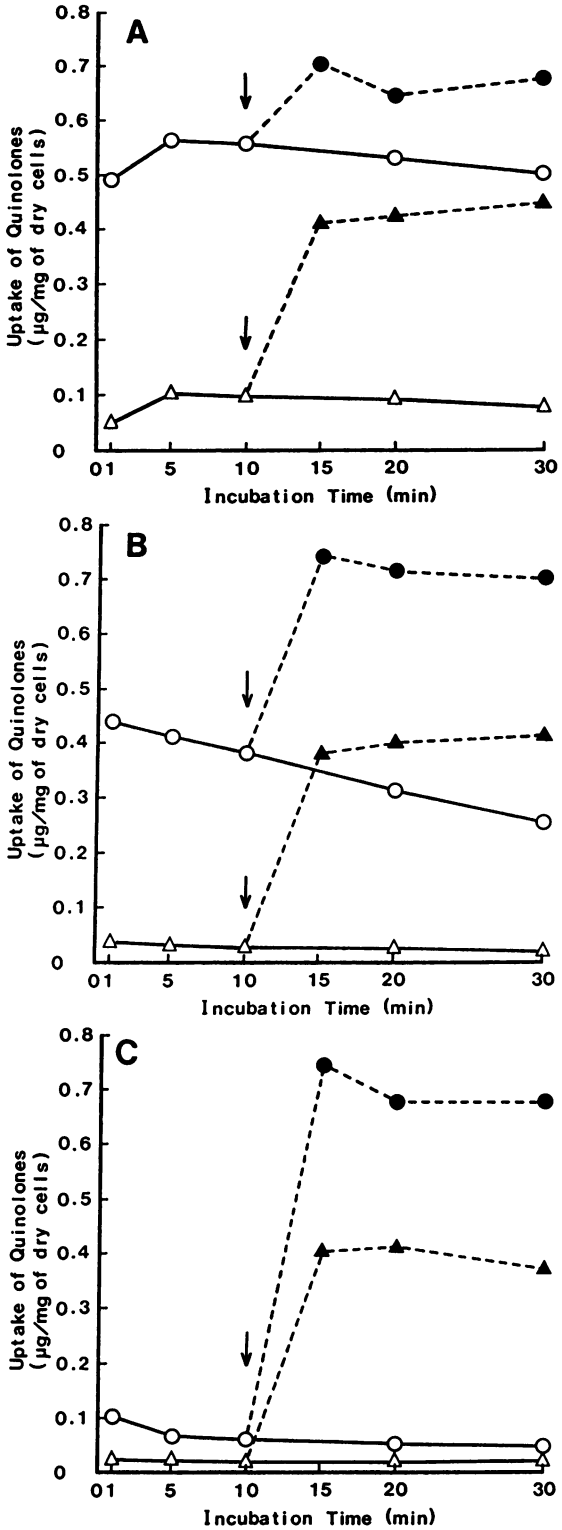

FIG. 2. Uptake of sparfloxacin and norfloxacin by *S. aureus* MS16008 (A), MS16401 (B), and NMS54 (C). Symbols: ○, sparfloxacin; △, norfloxacin; ●, sparfloxacin with CCCP; ▲, norfloxacin with CCCP. CCCP was added at the time indicated by the arrow.

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