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Letter to the Editor

Inhibition of the Multidrug Transporter NorA Prevents Emergence of Norfloxacin Resistance in *Staphylococcus aureus*

Fluoroquinolone antibiotics effectively kill *Staphylococcus aureus*, but their clinical use against this pathogen is limited by the rapid emergence of resistant variants. The most common resistance mechanisms characteristic of clinical fluoroquinolone-resistant isolates of *S. aureus* involve mutations of topoisomerase IV (4) or DNA gyrase (2) which presumably reduce the affinity of these target enzymes for fluoroquinolones. The third mechanism, providing a somewhat lower degree of resistance and found mostly in the resistant strains selected in vitro, involves overexpression of a multidrug efflux transporter, NorA. NorA pumps a variety of toxic compounds, including fluoroquinolones, from the bacterial cell to the outer medium, thus reducing the cytoplasmic drug concentration (6–9).

Transcripts of the *norA* gene can be detected not only in resistant but also in fluoroquinolone-susceptible strains of *S. aureus* (5, 6). This level of NorA expression in wild-type *S. aureus* confers significant intrinsic resistance to fluoroquinolones. Kaatz and Seo have recently demonstrated that the plant alkaloid reserpine, which was shown previously to inhibit NorA (7), potentiates the bacteriocidal action of norfloxacin toward wild-type *S. aureus*; in the presence of reserpine the MIC of norfloxacin becomes four times smaller than in its absence (5). We have confirmed this result by showing that reserpine, which by itself does not affect the growth of *S. aureus*, potentiates the growth-inhibitory effect of norfloxacin towards the fluoroquinolone-susceptible strain of *S. aureus* SA1199 (kindly provided by G. W. Kaatz, Wayne State University, Detroit, Mich.): the 50% inhibitory concentration of norfloxacin in the presence of reserpine is four to five times lower than in its absence (Fig. 1).

Furthermore, we have found that reserpine reduces the rate of emergence of norfloxacin-resistant variants among wild-type *S. aureus* cells. SA1199 cells were mutagenized by treatment with ethyl methanesulfonate (1); grown in Luria-Bertani (LB) medium; and, at the logarithmic phase of growth, plated on LB agar (10^8 cells per plate) at different concentrations of norfloxacin, either in the presence or in the absence of 20 μg of reserpine per ml. The resistant colonies appearing on the plates were counted after 48 h of incubation at 37°C. The results of two independent experiments, shown in Table 1, demonstrate that reserpine dramatically suppresses the emergence of norfloxacin-resistant colonies. The results obtained with nonmutagenized *S. aureus* were similar, but the absolute numbers of resistant colonies were approximately 1 order of magnitude lower (data not shown). It should be noted that in the absence of norfloxacin, reserpine has very little, if any, effect on the plating efficiency of *S. aureus*. When plated on LB or LB supplemented with 20 μg of reserpine per ml, SA1199 cells (approximately 10^3 cells per plate; five plates in each case) produced 997 ± 89 and 889 ± 113 colonies, respectively, with no detectable difference in the colony size.

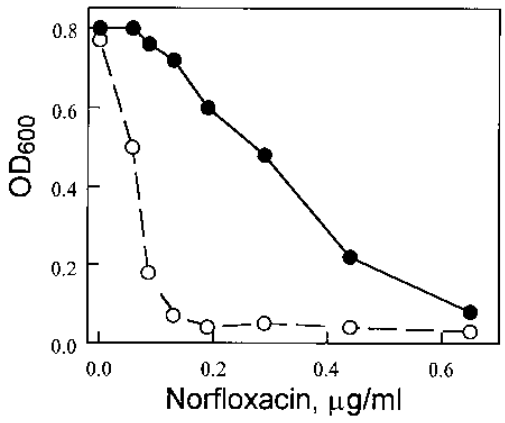

FIG. 1. Effect of reserpine on susceptibility of *S. aureus* SA1199 to norfloxacin. Cells were inoculated into tubes with LB medium (2×10^5 cells per ml) containing different concentrations of norfloxacin and either no reserpine (filled circles) or 20 μg of reserpine per ml (open circles) and incubated with shaking at 37°C for 4.5 h. Optical densities (OD) of the cultures at the end of incubation are shown.

There are two possible explanations for the dramatic effect of reserpine on the emergence of norfloxacin resistance. One possibility is that the absolute majority of colonies growing on norfloxacin plates are resistant to the antibiotic because of NorA overexpression; reserpine inhibits NorA and thus prevents growth of such colonies. This explanation contradicts, however, recent results of Ferrero et al. (3) showing that mutations in the topoisomerase IV gene, *grlA*, are the major cause of resistance in the first steps of fluoroquinolone selection. Another, more likely explanation is that reserpine, by inhibiting NorA expressed in wild-type *S. aureus* cells, increases the intracellular concentration of the drug to such levels that most *grlA* mutations fail to protect cells from killing by the drug. Molecular analysis of the resistant colonies is necessary to discern between these two possibilities.

Regardless of the precise mechanism of the effect of reserpine, it appears that the clinical efficiency of fluoroquinolone antibiotics against *S. aureus* can be improved substantially by supplementing them with a NorA inhibitor. Unfortunately, reserpine is unsuitable for this purpose. Although it is a commonly used antihypertensive drug, reserpine is neurotoxic at the concentrations required to inhibit NorA. We are currently

TABLE 1. Number of norfloxacin-resistant colonies of *S. aureus* at different concentrations of the drug and effect of NorA inhibition by reserpine

Expt no.	No. of resistant colonies at norfloxacin concn (μg/ml):				
	1.25	2.5	5	10	20
1					
− Reserpine	1,508	173	48	29	0
+ Reserpine	72	21	1	0	0
2					
− Reserpine	1,888	495	155	48	
+ Reserpine	117	82	0	0	

searching for nontoxic inhibitors of NorA which potentially can be employed in clinical practice.

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Penelope N. Markham
Alexander A. Neyfakh
Department of Medicinal Chemistry and Pharmacognosy and Center for Pharmaceutical Biotechnology (M/C 870)
University of Illinois at Chicago
900 S. Ashland Ave.
Chicago, Illinois 60607