Uptake of Minocycline and Tetracycline by Tetracycline-Susceptible and -Resistant Bacteria

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Minocycline (7-dimethylamino-6-demethyl-6-deoxytetracycline) is a new semi-synthetic tetracycline with potent activity against tetracycline-susceptible bacterial pathogens and unique activity against tetracycline-resistant staphylococci. Studies to determine the basis for this unique activity showed that, whereas tetracycline-resistant staphylococci took up less ^{3}H-tetracycline than the susceptible cells, both the tetracycline-resistant and -susceptible cells accumulated equivalent amounts of ^{14}C-minocycline. In contrast, tetracycline-resistant *Escherichia coli* cells were relatively resistant to minocycline and accumulated less of both drugs than did the susceptible organisms. It is proposed that minocycline is effective against tetracycline-resistant staphylococci because of its ability to penetrate the cells sufficiently to reach inhibiting concentrations at sensitive reaction sites.

Minocycline (7-dimethylamino-6-demethyl-6-deoxytetracycline) is a new semisynthetic tetracycline with high activity against tetracycline-susceptible bacterial pathogens and with unique activity against tetracycline-resistant staphylococci [13]. It was highly active in mice with tetracycline-resistant staphylococcal infections that were unresponsive to treatment with large doses of doxycycline, methacycline, and demeclocycline [12, 16]. Its remarkable activity against resistant staphylococcal clinical isolates has been reported by several investigators [5, 7, 12, 15, 18, 20].

Several investigators have shown that tetracycline uptake is diminished in resistant strains [3, 6, 10, 17, 19]. This report deals with preliminary studies to determine whether uptake accounts for the activity of minocycline against tetracycline-resistant staphylococci. We compared the uptake of minocycline and tetracycline by tetracycline-susceptible and -resistant cells of *Staphylococcus aureus* and *Escherichia coli*.

MATERIALS AND METHODS

The tetracycline-susceptible *S. aureus* strain Smith (ATCC 13709) was compared with the tetracycline-resistant strain Rose (ATCC 14154). Also, *S. aureus* 8325 (80α), a susceptible strain, was compared with a resistant strain derived from it by transfer of an extrachromosomal tetracycline-resistance factor from strain E_{169}. These strains were kindly provided by R. P. Novick (Public Health Institute of the City of New York). The tetracycline-susceptible *E. coli* strain UC311 was compared with two tetracycline-resistant strains derived from it by transferring the resistance factor from two donor strains, *Salmonella* DY and *Shigella* RB, received from S. A. Kabins [11].

The uptake of the antibiotics was measured in viable and nonviable (heated in boiling-water bath for 15 min) cells from cultures grown in Penassay Broth (Difco) to mid-log phase. ^{14}C-minocycline (specific activity, 2.44 μCi/mg) or ^{3}H-tetracycline (specific activity, 10.55 μCi/mg) was added to portions of cultures at 37 C. The uptake was terminated after 20 min by rapidly filtering 1 ml of the reaction mixture through a membrane filter (Millipore Corp.; 25-mm type GS; pore size, 0.22 μm) that had been washed with ice-cold buffer (Sorensen's pH 7.2). The impinged cells were washed immediately with cold buffer (twice with 5 ml). The membranes were transferred to scintillation vials and dissolved in 5 ml of scintillation solution (0.5% Scintillator Butyl-PBD Ciba in methyl cellosolve-toluene, 1:1) and 5 ml of a suspension of Cab-O-Sil in the above scintillation solution, 1:1 by volume [14]. Counts were made for duplicate samples in a liquid scintillation spectrometer (model 3003, Packard Instrument Co., Inc.). The counts obtained with the killed cells were subtracted from the counts obtained with the viable cells to determine the uptake that is dependent on functional cell processes.

For determinations of the dry weight of cells, portions of cultures were centrifuged. The cells were washed with distilled water and dried to constant weight over P_2O_5 in vacuo.

RESULTS AND DISCUSSION

Both the tetracycline-susceptible and tetracycline-resistant staphylococcal strains were

TABLE 1. *Minimal inhibitory concentrations (MIC) of tetracycline and minocycline for staphylococcal and E. coli strains*

Strain	MIC (μg/ml)[a]	
	Tetracycline	Minocycline
S. aureus		
8325	0.03	0.03
8325 T$_{169}$	64	0.12
Smith	0.12	0.01
Rose	128	0.5
E. coli		
311	1	0.5
311-RB	128	16
311-DY	64	16

[a] Broth dilution method, Penassay Broth. Incubation: 37 C, 24 h.

highly susceptible to minocycline (Table 1). In all strains, equivalent amounts of minocycline were accumulated and uptake of minocycline was greater than that of tetracycline. Tetracycline-resistant cells accumulated less tetracycline than did tetracycline-susceptible staphylococci (Fig. 1).

Unlike the staphylococci, tetracycline-resistant *E. coli* strains were also relatively resistant to minocycline (Table 1). The tetracycline-resistant *E. coli* strains accumulated less minocycline as well as less tetracycline than did the tetracycline-susceptible cells. Each strain took up about as much minocycline as tetracycline (Fig. 2).

Our results with tetracycline are in agreement with those of other investigators who reported less accumulation of the antibiotic by resistant cultures. Reynard et al. (17) tested a large number of clinical isolates of *E. coli* and found that the uptake of tetracycline was lower in the resistant strains than in the susceptible strains. Sompolinsky et al. (19) reported that resistant *S. aureus* cells accumulated tetracycline to a significantly lower degree than susceptible strains. In our study, we found a significant difference in the accumulation of tetracycline between tetracycline-susceptible and -resistant staphylococci but no difference in the accumulation of minocycline.

The greater uptake of minocycline than of tetracycline by staphylococcal cells may be due to the lipophilicity of minocycline, which is greater (2) than that of tetracycline. Several investigators have suggested that there may be a relation between antibiotic resistance and the lipid content of bacterial cells (4, 8, 9). Dunnick and O'Leary (4) reported that an antibiotic-resistant strain of *S. aureus* contained more lipid than a susceptible strain. In contrast,
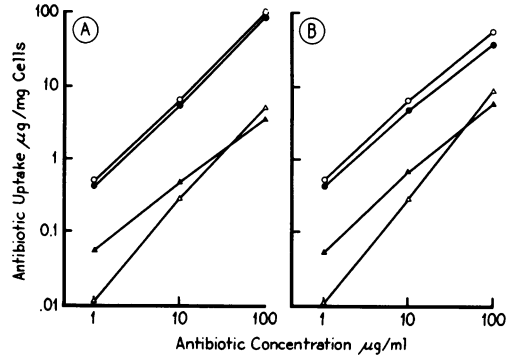

FIG. 1. *Uptake by staphylococci:* [14]*C-minocycline by tetracycline-susceptible (●) and resistant (○) strains;* [3]*H-tetracycline by susceptible (▲) and resistant (△) strains. (A) Comparison of tetracycline-susceptible strain 8325 and the resistant strain 8325-T$_{169}$ derived from the susceptible strain by the transfer of an extrachromosomal resistance factor. (B) Comparison of tetracycline-susceptible strain Smith and tetracycline-resistant strain Rose.*
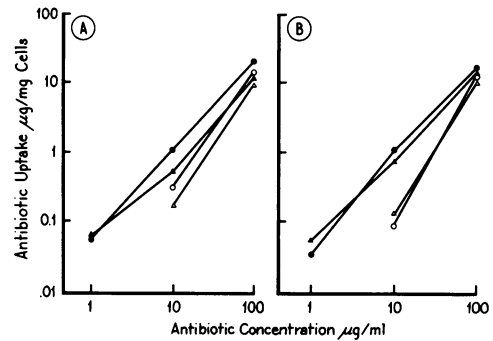

FIG. 2. *Uptake of E. coli:* [14]*C-minocycline by susceptible (●) and resistant (○) strains;* [3]*H-tetracycline by susceptible (▲) and resistant (△) strains. (A) Comparison of susceptible strain 311 and the resistant strain derived from it by transferring the resistance factor from donor strain Salmonella DY. (B) Comparison by strain 311 and the resistant strain derived from it by transferring the resistance factor from donor strain Shigella RB. Uptake at 1 μg/ml exposure was not detectable for resistant strains.*

susceptible and resistant gram-negative organisms had the same lipid content but a different composition of fatty acids. Blackwood and English (1) observed that, the greater the lipophilicity of a tetracycline analogue, the greater its in vitro activity against tetracycline-resistant staphylococci. The lipophilic quality of minocycline may enable it to be accumulated by the tetracycline-resistant staphylococcal cell with its high lipid content in sufficient quantity to effect inhibition of growth. In the case of *E. coli*, the lipophilicity may not be of special advan-

tage to affect its uptake since there is no difference in lipid content between tetracycline-susceptible and -resistant cells.

Our findings suggest that minocycline is effective against tetracycline-resistant staphylococci because of its ability to penetrate the cells sufficiently to reach inhibitory concentrations at the site of sensitive reactions.

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