

## In Vitro Activity of LY146032 Alone and in Combination with Other Antibiotics against Gram-Positive Bacteria

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**The antibacterial activity of LY146032 alone and in combination with other drugs was assayed against gram-positive isolates. Synergism was found when LY146032 was combined with netilmicin, amikacin, imipenem, and fosfomycin by both checkerboard and time-kill tests. Indifference predominated when LY146032 was combined with teicoplanin, vancomycin, and rifampin. Under no circumstances and with no combinations was an antagonistic effect detected.**

LY146032 is a newly developed cyclic lipopeptide belonging to the group of antimicrobial agents known as peptolides. Previous data (4-6) indicated that this antibiotic is active against a broad range of gram-positive bacteria by inhibiting cell-wall synthesis with a mechanism different from that of other available drugs. In addition, LY146032 demonstrated an excellent bactericidal effect on the strains tested in previous studies (4-6). This may be of special interest, because many cell-wall-active agents do not kill microorganisms such as enterococci (1, 7). On the other hand, in severe clinical infections caused by enterococci, the use of a single drug frequently leads to unacceptable failure rates (3). Thus, it becomes necessary to treat infections with an antibiotic combination, usually including an aminoglycoside and a cell-wall-active agent (9).

For these reasons, it may be of some consequence to know how LY146032 acts in combination with other drugs.

In this study, we evaluated the in vitro activity of LY146032 against gram-positive aerobic bacteria in comparison with that of netilmicin, amikacin, imipenem, fosfomycin, rifampin, teicoplanin, and vancomycin.

Test strains included 35 isolates of *Staphylococcus* spp. and 15 isolates of *Enterococcus* spp., all freshly isolated from clinical specimens and identified by conventional tests as previously described (2, 10).

The antibiotics were obtained as follows: LY146032, Eli Lilly Italia, Sesto Fiorentino, Italy; teicoplanin and rifampin, Gruppo Lepetit Spa, Milan, Italy; fosfomycin, Zambon Spa, Milan, Italy; and imipenem, Merck Sharpe & Dohme, Rome, Italy. The other drugs were obtained from commercial sources.

Sterile stock solutions of the antibiotics were prepared from the standard reference powders in accordance with the instructions of the manufacturers.

Test media containing LY146032 were supplemented with calcium chloride to achieve a final calcium content of 50 mg/liter, in accordance with the suggestions of Eliopoulos et al. (4).

The activity of LY146032 in combination with each of the other drugs was assayed by checkerboard titration on a total of 50 strains, and 20 strains were tested by the time-kill system with Mueller-Hinton broth as the test medium.

Checkerboard studies were performed in microdilution trays as previously described (2). Antibiotic interactions were determined based on the fractional inhibitory concen-

tration calculated by the following formula: MIC of antibiotic A in the presence of antibiotic B/MIC of antibiotic A alone + MIC of antibiotic B in the presence of antibiotic A/MIC of antibiotic B alone (8). Synergism is present if the fractional inhibitory concentration index is  $<0.5$ , and antagonism is present if the index exceeds 4. Indifference or additive effect is represented by an index of 0.5 to 4.

Time-kill studies were performed by adding the antibiotics to log-phase bacterial cultures diluted to  $10^6$  to  $10^7$  CFU/ml and growing in 500-ml flasks at 37°C. LY146032 and all other drugs were used at concentrations corresponding to half their MICs. Just before the antibiotics were added (zero time) and at 2, 6, and 24 h thereafter, the viable numbers of organisms were determined with serial 10-fold dilutions made in physiologic saline. A calibrated micropipette was used to deliver 100- $\mu$ l portions from each dilution onto a Mueller-Hinton agar plate. Because a drug carry-over effect was detected, especially when antibiotics were sampled in combination, only  $10^3$  CFU/ml could be accurately counted. Any data below this detectable level were not reported. Antibiotic interactions were interpreted as synergistic or antagonistic if the antibiotic combination, compared with the most effective single antibiotic, caused at least a 100-fold reduction or increase, respectively, in the CFU at 24 h. Intermediate results were interpreted as indifference.

The in vitro combination of LY146032 with netilmicin or amikacin resulted in a synergistic interaction for about 40% of the microorganisms by the checkerboard method. The percentage exceeded 75% if the time-kill system was used. Antagonism was never registered.

The in vitro combination of LY146032 with imipenem tested by the checkerboard method resulted in a synergistic interaction for all staphylococci tested and for 60% of *Enterococcus faecalis* isolates. With the time-kill method, this antibiotic combination was synergistic for all strains.

A high percentage of synergism (80%) was also found when LY146032 was added to fosfomycin and tested by the checkerboard method. When the time-kill system was used, the interaction resulted in almost complete synergism for all strains except a methicillin-resistant *Staphylococcus aureus* strain which showed indifference. Antagonism was not found.

On the contrary, with the combination of LY146032 and vancomycin or teicoplanin indifference was the prevalent response with both methods. Antagonism was never observed. With checkerboard titration, LY146032 combined with rifampin showed indifference for the great majority of

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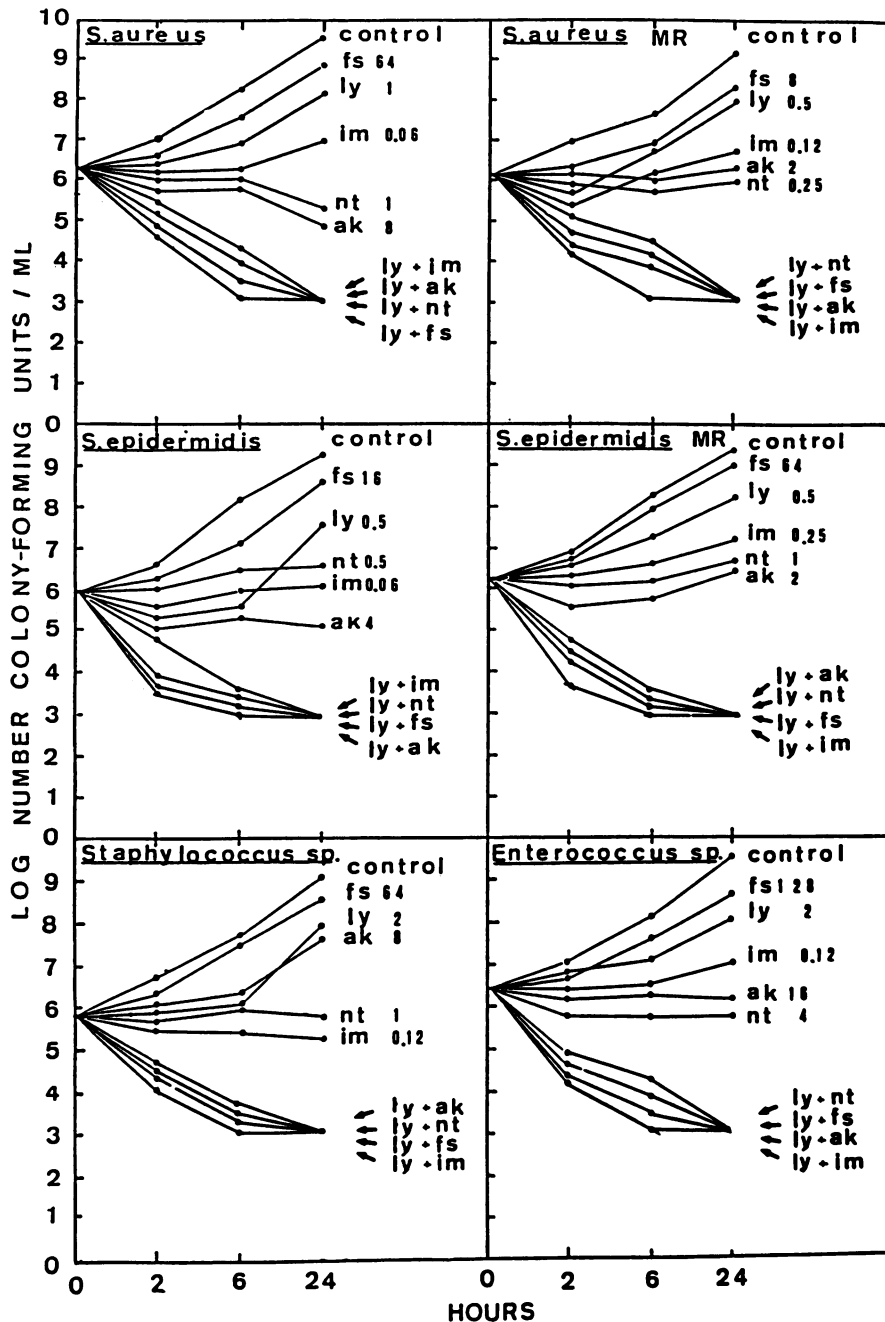


FIG. 1. Effects of various antibiotics alone and in combination against representative gram-positive strains. Time-kill tests were performed in the presence of the indicated drug concentrations (expressed in micrograms per milliliter). Abbreviations: ly, LY146032; fs, fosfomycin; im, imipenem; nt, netilmicin; ak, amikacin.

strains tested (82%). The same outcome was obtained in the time-kill system. In no instance was antagonism encountered. In Fig. 1 are reported the results of time-kill curves determined for representative pathogens.

As reported elsewhere (4-6), the novel lipopeptide antibiotic LY146032 possessed good antibacterial activity against all gram-positive bacteria tested. It is noteworthy that the pathogens were isolated from severe syndromes, such as endocarditis, septicemia, pneumonia, osteomyelitis, and soft tissue and urinary tract infections.

LY146032 reacted favorably in vitro in combination with all the antibiotics tested. Notably, the new compound

showed a high incidence of synergism when combined with the aminoglycoside antibiotics imipenem and fosfomycin, and in no instance was an antagonistic effect detected.

The great intrinsic antibacterial activity and the high incidence of synergism observed suggest that clinical trials with antibiotic combinations that include LY146032 are warranted.

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