Comparative In Vitro Activities of LY191145, a New Glycopeptide, and Vancomycin against \textit{Staphylococcus aureus} and \textit{Staphylococcus-Infected Fibrin Clots}

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Bactericidal activities of LY191145, an investigational glycopeptide, and vancomycin against \textit{Staphylococcus aureus} were evaluated. Only LY191145 at a concentration 16-fold greater than the MIC was able to achieve 99.9% killing against methicillin-susceptible \textit{S. aureus} (ATCC 25923; 8.0 \textmu g/ml). Both agents demonstrated 99.9% killing against methicillin-resistant clinical isolate \textit{S. aureus} MRSA67 over 24 h at concentrations 4, 8, and 16-fold greater than the MIC, but bacteria were killed at a more rapid rate by LY191145 (1.63 versus 5.02 h; \textit{P} < 0.001). Against strain ATCC 25923- and MRSA67-infected fibrin clots, total reductions by LY191145 and vancomycin over 72 h were not statistically significantly different at a concentration 16 times the MIC (1.12 ± 0.31 and 1.23 ± 0.13 and 1.40 ± 0.17 and 1.36 ± 0.37 CFU/g; respectively). Increasing the drug concentration to 50 times the MIC did not alter the values significantly, and there was no statistically significant difference between the two agents. Overall, LY191145 exhibited more rapid bactericidal activity than vancomycin against \textit{S. aureus}, and a concentration 16-fold greater than the MIC appears to be optimal.

LY191145 is a semisynthetic glycopeptide antibiotic derived from \textit{Amycolatopsis orientalis} (previously designated \textit{Nocardia orientalis} and \textit{Streptomyces orientalis}). It has a structure-activity relationship similar to that of vancomycin. This mono-\textit{N-p} chlorobenzyldervative demonstrates excellent in vitro activity against \textit{Staphylococcus aureus}, with a MIC of 0.25 to 0.5 mg/ml (6). It is also very potent against both vancomycin-susceptible and -resistant enterococci. The geometric mean MICs are 0.65 and 3.6 mg/ml, respectively (6).

We compared the in vitro activity of LY191145 with that of vancomycin at fractions and multiples of the MIC against two strains of \textit{S. aureus}. LY191145 susceptibility test grade powder (lot BL59NY184) was supplied by Lilly, Indianapolis, Ind., and vancomycin for injection (lot 121004; Lyphomed, Deerfield, Ill.) was purchased commercially. Study strains included a reference strain of methicillin-susceptible \textit{S. aureus} (ATCC 25923) and a clinical isolate of methicillin-resistant \textit{S. aureus} (MRSA67) recovered from a patient admitted to the Detroit Medical Center.

The MICs and MBCs of LY191145 and vancomycin were determined by a microtiter broth dilution method with inocula of \textit{5 × 10}^6 and \textit{5 × 10}^7 CFU/ml by following National Committee for Clinical Laboratory Standards guidelines (7). The test medium was Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with calcium and magnesium (25 and 12.5 mg/liter, respectively). MIC and MBC tests were also repeated in the presence of 4% human albumin (lot HA1111; Armour, Collegeville, Pa.). Test tube killing curves were conducted by using an inoculum of approximately \textit{10}^5 CFU of \textit{S. aureus} per ml at drug concentrations of 0.25, 0.5, 1, 2, 4, 8, and 16 times the MIC. All experiments were performed in duplicate over 24 h. Samples (0.1 ml) were removed at 0, 2, 4, 8, and 24 h, and after appropriate dilution with 0.9% sodium chloride, 20 ml was plated on tryptic soy agar (Difco) in triplicate. Plates were then incubated at 37°C for 24 h, and the colonies were counted thereafter. An antibiotic carryover experiment was conducted to identify drug concentrations that could potentially affect the colony counts. Potential drug carryover samples (0.1 ml) were placed in 10 ml of 0.9% sodium chloride and filtered with a 0.45-\textmu m-pore-size Millipore filter system. Filters were then placed aseptically on tryptic soy agar and incubated for 24 h. The reliable limit of detection in our laboratory has been determined to be 100 CFU/ml (5). Time-kill curves over 24 h were constructed as \textit{log}_{10} CFU per milliliter versus time. The time required to achieve a 99.9% reduction and the total reduction in \textit{log}_{10} CFU per milliliter over 24 h were determined.

Fibrin clots of approximately 1 ml and 1 g were prepared as previously described (4, 5), by mixing 0.8 ml of human cryoprecipitate antihemolytic factor from volunteer donors (lot M92118051; American National Red Cross, Detroit, Mich.) and a pellet (0.1 ml) of \textit{staphylococci} in a sterile, siliconized 1.5-ml Eppendorf tube. Bacterial inocula were prepared by inoculating two or three colonies into 10 ml of supplemented Mueller-Hinton broth, which was then incubated at 37°C for 24 h on a rotator. After centrifugation for 15 min at 3,500 \times g and 25°C, the supernatant was removed. Each pellet consisted of approximately \textit{10}^{10} CFU/0.1 ml. Sterile monofilament line was placed into the cryoprecipitate-bacterium mixture. Bovine thrombin (5,000 \textmu U; lot 00323P; Parke-Davis, Morris Plains, N.H.) was reconstituted with 5.0 ml of sterile calcium chloride (50 mmol), and 0.1 ml of the thrombin was then added to the cryoprecipitate-bacterium mixture (10). This gelatinous mixture was then removed from the Eppendorf tube by using a sterile 21-gauge needle. Killing curves were determined with the infected fibrin clots suspended in 10 ml of supplemented

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Mueller-Hinton broth over 72 h. The optimal concentration, based on the preliminary killing curves obtained with multiples of the MIC, was used to evaluate the bactericidal activities of LY191145 and vancomycin against S. aureus-infected fibrin clots with an inoculum of approximately $5 \times 10^5$ CFU/g. All experiments were performed in duplicate over 72 h. Two fibrin clots were removed at 0, 24, 48, and 72 h. The fibrin clots were weighed and placed in a 2-ml sterile capped vial prefilled with 3-mm-diameter glass beads, 0.5 ml of 1.25% trypsin (1:250 powder; lot 17404; Difco), and 0.5 ml of 0.9% sodium chloride. To homogenize the clot, the vial was placed in a minibead beater grinder (Biospec Products, Bartlesville, Okla.) for 30 s. Dilutions were made as previously described, and the colonies were counted after incubation. Time-kill curves determined over 72 h were plotted as $\log_{10}$ CFU per g versus time. The time required to achieve a 99.9% reduction, determined by linear regression, and the total reduction in $\log_{10}$ CFU per g over 72 h were assessed with a two-way analysis of variance and Tukey's test. $P$ values of $<0.05$ were considered significant.

The MICs and MBCs of LY191145 and vancomycin against ATCC 25923 and MRSA67 at an inoculum of $5 \times 10^5$ CFU/ml were 0.39 and 0.78, 0.78 and 1.56, 0.098 and 0.098, and 0.39 and 0.39 $\mu$g/ml, respectively. Both agents demonstrated a significant inoculum effect; the corresponding values for $5 \times 10^7$ CFU/ml were 3.125 and 6.25, 3.126 and 12.5, 1.56 and 1.56, and 3.125 and 6.25 $\mu$g/ml, respectively. The MICs and MBCs of LY191145 and vancomycin in the presence of 4% albumin against ATCC 25923 and MRSA67 were 0.39 and 3.13, 0.78 and 3.13, 0.098 and 0.098, and 0.78 and 1.56 $\mu$g/ml, respectively. In the time-kill experiments, ATCC 25923 regrew with LY191145 at concentrations 0.25, 0.5, and 1 times the MIC whereas there was no difference in bactericidal activity between concentrations 2, 4, and 8 times the MIC of LY191145. A LY191145 concentration 16-fold greater than the MIC demonstrated the most rapid bactericidal activity of all of the concentrations tested, and this was the only concentration able to achieve a 99.9% reduction in colony counts in approximately 8.0 h (Fig. 1A). Vancomycin at concentrations 0.25 and 0.5 times the MIC did not demonstrate any killing activity against ATCC 25923 (Fig. 1B). Although there was no difference in activity between concentrations 1, 2, 4, 8, and 16 times the MIC during the first 8 h, at the MIC, there was breakthrough growth similar to the growth curve. A concentration twofold greater than the MIC demonstrated no further reduction in colony counts after the first 8 h. Finally, there was no significant difference in overall killing activity between concentrations 4, 8, and 16 times the MIC over 24 h. Overall, a 3-log reduction was not achieved with any of the vancomycin concentrations. Against MRSA67, both LY191145 and vancomycin concentrations 4, 8, and 16 times the MIC achieved a 99.9% reduction over 24 h. Concentrations 8 and 16 times the MIC of LY191145 required less time than a concentration 4 times the MIC to achieve a 3-log reduction (1.65, 1.63, and 3.46 h; $P < 0.017$) (Fig. 2A). Although there was some killing activity at concentrations 0.25, 0.5, 1, and 2 times the MIC of LY191145, breakthrough growth was noted at all of these concentrations. There was killing activity at concentrations 0.25, 0.5, and 1 times the MIC of vancomycin against MRSA67 (Fig. 2B). Breakthrough growth occurred with a concentration twice the MIC in two of four test tubes. Vancomycin demonstrated statistically significantly more killing activity at a concentration FIG. 1. Killing curves of methicillin-susceptible S. aureus (ATCC 25923) exposed to various concentrations of LY191145 (A) and vancomycin (B). FIG. 2. Killing curves of methicillin-resistant S. aureus (MRSA67) exposed to various concentrations of LY191145 (A) and vancomycin (B).
The average starting inoculum of the infected fibrin clots was 9.49 ± 0.22 log_{10} CFU/g. On the basis of time-kill experiments, the concentration 16-fold greater than the MIC of each agent was chosen as the optimal concentration and was used for killing curve experiments with infected fibrin clots. Against ATCC 25923- and MRSA67-infected fibrin clots, total reductions in CFU per gram over 72 h were not statistically significantly different between LY191145 and vancomycin at a concentration 16 times the MIC (1.12 ± 0.31, 1.23 ± 0.13, 1.40 ± 0.17, and 1.36 ± 0.37 CFU/g) (Table 1). In fibrin clot experiments, concentrations 50 times the MICs of LY191145 (15 μg/ml) and vancomycin (30 μg/ml) were also tested against S. aureus-infected fibrin clots, and there was no statistically significant difference between the two agents in terms of total reduction in vegetation titer over 72 h.

Some investigators have shown that glycopeptide and β-lactam antibiotics possess concentration-independent killing activity (1–3, 8). In as to those studies, we have found that the bactericidal activity was not enhanced by increasing concentrations of LY191145 and vancomycin. Against methicillin-susceptible and -resistant S. aureus, both LY191145 and vancomycin at concentrations 4-, 8-, and 16-fold greater than the MICs demonstrated similar degrees of bactericidal activity.

Although the bactericidal activity of LY191145 was statistically significantly greater than that of vancomycin against both strains of S. aureus at an inoculum of 5 × 10^2 CFU/ml, LY191145 was as potent as vancomycin against infected fibrin clots. Furthermore, the total reduction in the vegetation titer over 72 h ranged between 1 to 2 logs for both agents. This may be due to the inoculum effect that was seen with the increase in the MIC at a high inoculum concentration (5 × 10^2 CFU/ml), as well as the effects of protein binding. Other investigators have shown that the potency of highly protein-bound glycopeptides such as teicoplanin (>90% killing) against gram-positive organisms is affected in the presence of protein (2, 3).

It is possible that some of the activity of LY191145 was affected by the presence of protein since it has been shown to be approximately 73% protein bound in rat serum (9). The MICs and MBCs of LY191145 for both strains of S. aureus did increase slightly in the presence of 4% human albumin in this experiment. Furthermore, the 72-h experimental time frame may not have been sufficient for significant bactericidal activity against these organisms in a metabolically reduced state. In conclusion, LY191145, an investigational glycopeptide, appears to demonstrate potent bactericidal activity against both methicillin-susceptible and -resistant S. aureus strains. However, its bactericidal activity may be hindered by pharmacodynamic factors such as the inoculum effect and the protein-binding effect. Further in vitro and in vivo studies are needed to further characterize the clinical significance of these effects.

### REFERENCES