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NOTES

Lysostaphin Treatment of Experimental Aortic Valve Endocarditis Caused by a *Staphylococcus aureus* Isolate with Reduced Susceptibility to Vancomycin

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The rabbit model of endocarditis was used to test the effectiveness of vancomycin and two different lysostaphin dosing regimens for the treatment of infections caused by a *Staphylococcus aureus* strain with reduced susceptibility to vancomycin (glycopeptide-intermediate susceptible *S. aureus* [GISA]). Vancomycin was ineffective, with no evidence of sterilization of aortic valve vegetations. However, rates of sterilization of aortic valve vegetations were significantly better for animals treated with either a single dose of lysostaphin (43%) or lysostaphin given twice daily for 3 days (83%) than for animals treated with vancomycin. Rabbits given a single dose of lysostaphin followed by a 3-day drug-free period had mean reductions in aortic valve vegetation bacterial counts of 7.27 and 6.63 $\log_{10}$ CFU/g compared with those for untreated control rabbits and the vancomycin-treated group, respectively. We conclude that lysostaphin is an effective alternative for the treatment of experimental aortic valve endocarditis caused by a clinical VISA strain.

Lysostaphin is a 27-kDa peptidase that is produced by *Staphylococcus simulans* and that specifically cleaves the pentaglycine cross-links unique to the cell wall of *Staphylococcus aureus* (11, 16). The antimicrobial properties of lysostaphin were studied in the 1960s and 1970s, but it was never developed as a therapeutic agent (3–6, 12–14). Recently, we reexamined the therapeutic potential of lysostaphin in the treatment of staphylococcal infection using the rabbit model of experimental aortic valve endocarditis (2). In the treatment of experimental endocarditis caused by methicillin-resistant *S. aureus*, lysostaphin demonstrated excellent bactericidal activity, with a significant reduction in mean aortic valve vegetation counts of 8.5 $\log_{10}$ CFU/g compared with that for the control group (2). With the recent emergence of *S. aureus* strains with reduced susceptibility to vancomycin (glycopeptide-intermediate susceptible *S. aureus* [GISA]) (7, 10, 15), we decided to study the in vitro and in vivo activities of lysostaphin against a clinical isolate of VISA.

Lysostaphin MICs were determined by the broth microdilution method in cation-adjusted Mueller-Hinton broth (Becton Dickinson, Cockeysville, Md.) according to the standards of the National Committee for Clinical Laboratory Standards (8), with a final inoculum of 10^5 CFU/ml. Bovine serum albumin (0.1%; Sigma) was added to prevent the adsorption of lysostaphin to polystyrene microtiter wells.

The rabbit model of aortic valve endocarditis, which has been described previously (1, 2, 9), was used to evaluate the antibiotic treatment regimens. A total of 33 female White New Zealand rabbits weighing 2 to 3 kg were injected intravenously through the marginal ear vein with 1 ml of an overnight culture containing 10^7 CFU of VISA HIP5827 per ml. HIP5827 is a clinical isolate recovered from a patient in Michigan in 1996. The vancomycin MIC for this isolate is 8 μg/ml and was kindly provided by Fred Tenover, Centers for Disease Control and Prevention (15).

Rabbits were assigned to one of four different treatment groups: (i) the control group, which received no treatment, (ii) a group that received lysostaphin (30 mg/kg of body weight twice a day [b.i.d.]) intravenously for 3 days, (iii) a group that received a single intravenous dose of lysostaphin (100 mg/kg) followed by a 3-day drug-free period, or (iv) a group that received the standard intravenous vancomycin dosage (30 mg/kg b.i.d.) for 3 days. The vancomycin dosage of 30 mg/kg b.i.d. was chosen because it has been shown to achieve peak levels similar to those observed in humans (1). Recombinant lysostaphin was supplied by AMBI, Inc., Purchase, N.Y., and was stored at 4°C. Fresh solutions were prepared daily in 0.05 M Tris HCl–0.145 M NaCl. Vancomycin was obtained from Abbott Laboratories, Chicago, Ill.

Four days after infection and at least 18 h after administration of the last dose of antibiotics to the 3-day treatment groups, the rabbits were killed and their hearts and kidneys were aseptically removed. Aortic valve vegetations and kidney abscesses or infarcts were removed, weighed, homogenized in saline, and serially diluted. Dilutions were plated on Mueller-Hinton agar, and the plates were incubated at 37°C for 48 h. Titers of bacteria were expressed as $\log_{10}$ CFU per gram of tissue. Sterile aortic vegetation cultures contained ≤ 2 $\log_{10}$ CFU/g, and sterile kidneys contained ≤ 1 $\log_{10}$ CFU/g (the limit of detection). The mean numbers of bacteria per gram of vegetation and kidney tissue in all treatment groups were compared by analysis of variance. The Student-Newman-Keuls test was used to adjust for multiple comparisons. For analysis of the rate of sterilization, we used Fisher's exact test (two-tailed)

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TABLE 1. Outcome of 3-day treatment of experimental GISA aortic valve endocarditis with lysostaphin

Regimen	No. of rabbits sterile at the following site/total no. of rabbits:		Mean ± SD $\log_{10}$ CFU/g of tissue	
	Valve vegetation	Kidney	Valve vegetation	Kidney
None (controls)	0/11	0/11	10.3 ± 0.51	7.46 ± 0.6
Vancomycin, b.i.d.	0/9	0/9	9.66 ± 1.1	3.14 ± 1.39[a]
Lysostaphin, b.i.d.	5/6[b]	4/6[c]	2.03 ± 0.06[d]	2.09 ± 2.2[a]
Lysostaphin, single dose	3/7	7/7[b]	3.03 ± 1.52[d]	1.0 ± 0.0[d]

[a] $P < 0.05$ compared to control group alone (Student-Newman-Keuls test).
[b] $P < 0.05$ compared to control group and vancomycin group (Fisher's exact test).
[c] $P < 0.05$ compared to control group (Fisher's exact test).
[d] $P < 0.05$ compared to control group and vancomycin group (Student-Newman-Keuls test).

with the permutation-style adjustment for multiple comparisons. A P value of <0.05 was considered statistically significant for all tests.

The MICs of antibacterial agents for HIP5827 were as follows: oxacillin, >500 μg/ml; vancomycin, 8 μg/ml; and lysostaphin, 0.0039 μg/ml. The results obtained from the 3-day antibiotic treatment regimen for experimental endocarditis caused by HIP5827 are presented in Table 1. Control rabbits had a mean ± standard deviation aortic valve vegetation bacterial count of 10.3 ± 0.51 $\log_{10}$ CFU/g, which is comparable to those reported previously from trials of endocarditis caused by methicillin-resistant *S. aureus* (1). As expected, vancomycin given twice daily was ineffective in treating experimental aortic valve endocarditis, with a mean aortic valve vegetation bacterial count reduction of 0.64 $\log_{10}$ CFU/g compared with that for the untreated control group.

Lysostaphin treatment by both regimens was very effective. The group dosed b.i.d. had mean aortic valve vegetation bacterial count reductions of 8.27 $\log_{10}$ CFU/g ($P < 0.05$) and 7.63 $\log_{10}$ CFU/g ($P < 0.05$) compared with those for the untreated control and vancomycin groups, respectively. The group that received a single dose of lysostaphin had similar results, with reductions in mean aortic valve vegetation bacterial counts of 7.27 $\log_{10}$ CFU/g ($P < 0.05$) and 6.63 $\log_{10}$ CFU/g ($P < 0.05$) compared with those for the untreated control and vancomycin groups, respectively. There was no statistical difference in the counts between the two lysostaphin treatment groups. Colonies of bacteria isolated from vegetation material from rabbits treated with lysostaphin underwent broth microdilution susceptibility testing, and the lysostaphin MIC for these strains was not increased compared to that for the untreated parent strain.

Rates of sterilization of aortic valve vegetations were also better for the two lysostaphin treatment groups than for either the vancomycin group or the untreated control group. Rates of sterilization of aortic valve vegetations were 83% for the group that received lysostaphin b.i.d. ($P = 0.02$ versus the vancomycin group) and 43% for the group that received a single dose of lysostaphin ($P = 0.06$ versus the vancomycin group). None of the rabbits treated with vancomycin had sterile aortic valve vegetations. The difference in rates of sterilization between the two lysostaphin treatment groups was not statistically significant ($P = 0.2$).

Table 1 also shows the results of kidney tissue cultures for the different treatment groups. Both lysostaphin dosing regimens resulted in statistically significant reductions in bacterial counts in kidney tissue compared with those for the untreated control group ($P < 0.05$). Rates of sterilization of kidney tissue bacterial cultures were 66.6% for the group that received lysostaphin b.i.d. ($P = 0.01$ versus the vancomycin group) and 100% for the group that received a single dose of lysostaphin ($P = 0.0001$ versus vancomycin). The difference between the two lysostaphin treatment groups was not statistically significant ($P = 0.1$).

Treatment of the rabbit model of experimental aortic valve endocarditis caused by GISA HIP5827 was unsuccessful following vancomycin monotherapy. In contrast, two different dosing regimens of lysostaphin produced significant numbers of sterile aortic valve vegetations and a significant reduction in bacterial counts in both aortic valve vegetations and kidney tissues. More importantly, administration of a single intravenous dose of lysostaphin, followed by a 3-day antibiotic-free period, sterilized 43% of aortic valve vegetations. Our results suggest that intravenous lysostaphin is an effective alternative for the treatment of aortic valve endocarditis caused by a clinical GISA strain. These results support further studies of the clinical use of lysostaphin for the treatment of severe human infections caused by *S. aureus* strains with reduced susceptibility to vancomycin.

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