

Activity of Glycopeptides against *Staphylococcus aureus* Infection in a Rabbit Endocarditis Model: MICs Do Not Predict In Vivo Efficacy

Nathalie Asseray, Cedric Jacqueline, Virginie Le Mabeccque, Eric Batard,
Denis Bugnon, Gilles Potel, and Jocelyne Caillon*

Laboratoire Antibiotologie, UPRES EA 3826, Nantes, France

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The in vivo efficacy of vancomycin and teicoplanin against five *Staphylococcus aureus* strains with different susceptibilities to them and methicillin was studied. Rabbits were allocated at random to groups for endocarditis induction with one of these five strains and then treated for 2 days with vancomycin or teicoplanin. Each treated group was compared with a control group infected with the same strain. Vancomycin and teicoplanin showed similar activities. Low MICs did not predict better in vivo results.

Glycopeptides constitute the drugs of reference for treating infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) strains, particularly severe septicemia or endocarditis (18). Two factors limit the clinical usefulness of glycopeptides. First, *S. aureus* strains with reduced susceptibility to glycopeptides have emerged. The first vancomycin-resistant strain was identified in Japan (11), and since then vancomycin resistance has been documented in Europe (3, 6, 20). Second, treatment failures despite in vitro susceptibility have been reported, most notably for patients with endocarditis and other severe infections (7, 19). Furthermore, new agents have been introduced recently for the treatment of staphylococcal infections. These facts warrant a reappraisal of the role for glycopeptides in the first-line treatment of severe *S. aureus* infections.

The objective of the study reported here was to evaluate the in vivo efficacy of glycopeptides in animals with severe staphylococcal infections due to strains with various patterns of susceptibility to methicillin and glycopeptides.

We studied five *S. aureus* strains, of which two strains were susceptible to both methicillin and glycopeptides (methicillin-susceptible *S. aureus* [MSSA] strains MSSA 1 and MSSA 2), two strains were resistant to methicillin but susceptible to glycopeptides (MRSA 3 and MRSA 4), and one strain was resistant to methicillin and exhibited heterogeneous reduced susceptibility to glycopeptides (glycopeptide-intermediate *S. aureus* [GISA] strain GISA 5). The four glycopeptide-susceptible strains were isolated from blood cultures, and the GISA strain was isolated from sputum of a cystic fibrosis patient. The *mecA* gene was detected by PCR in strains MRSA 3, MRSA 4, and GISA 5. MICs of vancomycin (Dakota pharm, Le Plessis-Robinson, France) and teicoplanin (Aventis, Paris, France) were determined by using the broth and agar dilution methods, with inoculum sizes ranging from 10^6 to 10^9 CFU/ml to look for a potential inoculum effect. Bactericidal activity was assessed based on the determination of minimal bactericidal concentrations (MBCs) by microdilution method and on the killing kinetics with an inoculum of 10^7 CFU/ml and 0, 1, 4, 8, and 20 mg

of vancomycin or teicoplanin/liter; bacteria were counted after 0, 6, 24, and 48 h.

The rabbit aortic valve endocarditis model was used for the in vivo studies (16). Endocarditis of the aortic valve and left ventricle was induced by introduction of a polyethylene catheter followed 24 h later by an intravenous injection of 10^8 CFU of *S. aureus*. For each *S. aureus* strain, there were three groups, namely, vancomycin, teicoplanin, and control, for a total of 15 groups of animals. On day 3, the controls were killed, and the animals in the two other groups were started on a 48-h course of vancomycin or teicoplanin. Vancomycin was given as a continuous infusion in a dose of 100 mg/kg of body weight/day so that the steady-state serum level was equivalent to the usual target in humans (at least 20 mg/liter). Teicoplanin was given as an intravenous infusion in a dose of 18 mg/kg/day in order to produce steady-state serum levels similar to that of vancomycin. Because teicoplanin has a long half-life, a bolus of 3 mg/kg was given before the continuous infusion. Serum assays of vancomycin and teicoplanin were performed during treatment with an immunoenzymetric method. The treated animals were killed on day 5.

The aortic vegetations were harvested, weighed, and used for quantitative cultures on agar for 24 h at 37°C. Bacterial counts were expressed as \log_{10} CFU per gram of vegetation.

Statistics. The primary evaluation criterion was the bacterial count in vegetation cultures (\log_{10} CFU per gram of vegetation). The mean \pm standard deviation count was determined for each group of animals. Analysis of variance (ANOVA) was used first to evaluate counts for each *S. aureus* strain. When ANOVA showed a significant difference, Scheffe's test was used for pairwise comparisons. All statistical tests were run on Statview (Abacus Concepts, Berkeley, Calif.).

Vancomycin and teicoplanin had MICs and MBCs (Table 1)

TABLE 1. MICs and MBCs of the five studied strains

Drug	MIC/MBC (mg/liter) for:				
	MSSA 1	MSSA 2	MRSA 3	MRSA 4	GISA 5
Vancomycin	0.5/0.5	1/1	1/1	0.5/1	4/8
Teicoplanin	0.25/0.25	0.5/1	1/1	0.5/1	8/12

* Corresponding author. Mailing address: Lab. Antibiotologie Clinique et Experimentale, UPRES EA 3826, Faculté de Médecine, 1 rue Gaston Veil, 44035 Nantes, France. Phone: (33) 2 40 41 28 54. Fax: (33) 2 40 41 28 54. E-mail: jcaillon@chu-nantes.fr.

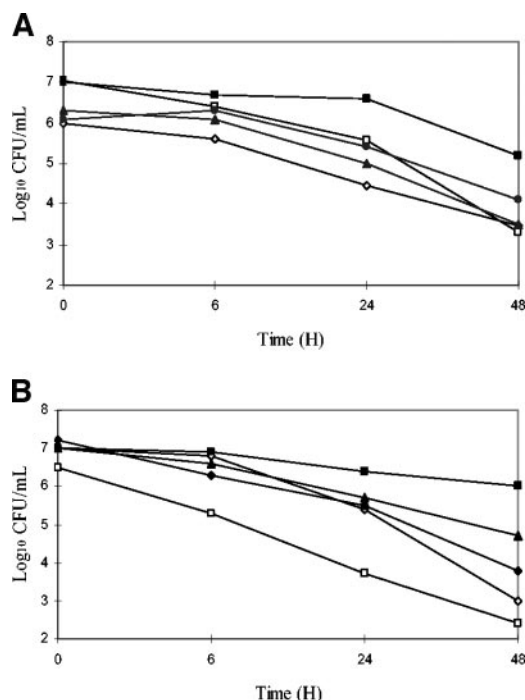


FIG. 1. Kill curves of vancomycin (A) and teicoplanin (B) at 20 mg/liter. \diamond , MSSA 1; \square , MSSA 2; \blacktriangle , MRSA 3; \bullet , MRSA 4; and \blacksquare , GISA 5.

ranging from 0.25 to 1 mg/liter for MSSA 1, MSSA 2, MRSA 3, and MRSA 4, indicating good in vitro susceptibility of these four strains to glycopeptides (NCCLS recommendations). No inoculum effect was found. For GISA 5, the MICs of vancomycin and teicoplanin were 4 and 8 mg/liter, respectively, confirming the reduced susceptibility of this strain to glycopeptides. Killing curves showed that vancomycin exhibited similar activities against all four glycopeptide-susceptible strains, whatever the concentrations used, contrasting with a marked decrease in bactericidal activity against the GISA 5 strain (Fig. 1A). With teicoplanin at the same concentrations, bactericidal activity against the two MRSA strains was decreased compared to that against the two MSSA strains, and an even greater decrease was noted with the GISA 5 strain (Fig. 1B).

The in vivo study (Table 2) showed that the two glycopeptides were active against only two of the five strains, namely, MSSA 1 and MRSA 3. With MSSA 2, a small but significant difference was noted compared to the control group. With MRSA 4 and GISA 5, no significant differences were found with the control group. For none of these five strains was a significant difference in activity noted between vancomycin and

teicoplanin. Levels of both glycopeptides in serum reached 20 mg/liter after 4 h and 30 mg/liter after 24 h.

The variability in glycopeptide activity noted in the rabbit endocarditis model is probably relevant to published reports of failed glycopeptide therapy in humans with staphylococcal infections. In these patients (7, 19), the absence of a therapeutic effect was not correlated with MIC elevation. Other agents or combinations of agents have been introduced recently for the treatment of staphylococcal infections, including those due to MRSA strains. Studies of the same rabbit model have shown early and reproducible activity of these new agents against several *S. aureus* strains, some of which were MRSA strains (1, 12).

The two glycopeptides used in our study were similar to each other regarding activity against the five *S. aureus* strains tested. Steady-state serum teicoplanin levels were far greater than 10 times the MIC for susceptible strains. This condition would be expected to ensure optimal efficacy, according to relevant data in the literature (2, 10, 14). The poor diffusion of teicoplanin within vegetations (4) and the high rate of protein binding (2) do not seem to have noticeably affected the level of activity against *S. aureus* in comparison with vancomycin.

Our in vivo data show that a low MIC does not always predict a better response to glycopeptides over the first 2 days of treatment. Early in vivo effects do not seem to be influenced by in vitro parameters.

Tolerance, defined as a loss of bactericidal activity (7, 13, 21), has been reported. We found no evidence of tolerance, as the in vitro bactericidal effect of vancomycin on the glycopeptide-susceptible strains was unimpaired. In contrast, the high MIC for the GISA 5 strain, classified as having intermediate susceptibility to glycopeptides, was strongly correlated with the loss of bactericidal activity in vitro and in vivo. This finding is in agreement with a study comparing two isogenic *S. aureus* strains with different glycopeptide susceptibility patterns in an endocarditis model (15).

Although the treatment period was brief (48 h) and the antibiotics had slow killing kinetics, we found noticeable differences in activity across strains that were not predicted by the in vitro data. The source of these differences must therefore be sought elsewhere than in the intrinsic in vitro activity of the antibiotics. Host-related factors can influence in vivo activity. Studies have investigated the bactericidal effects of endogenous peptides produced by neutrophils or platelets that seem to act by causing lysis of the bacterial wall (23, 24). Their activity may be influenced by exposure to some antibiotics, most notably those with effects on the bacterial wall, such as penicillins and vancomycin (22). Conceivably, an interaction

TABLE 2. In vivo results after 48-h treatment of the five studied strains

Regimen	Mean log ₁₀ CFU/g of vegetation \pm SD (no. of rabbits)				
	MSSA 1	MSSA 2	MRSA 3	MRSA 4	GISA 5
Controls	7.3 \pm 1.7 (6)	9.7 \pm 0.9 (5)	8.7 \pm 0.9 (5)	8.4 \pm 1.4 (8)	8.2 \pm 1.1 (4)
Vancomycin	3.0 \pm 1.3 (7) ^a	8.3 \pm 1.5 (5)	3.0 \pm 0.9 (4) ^a	6.6 \pm 1.6 (6)	6.7 \pm 1.9 (6)
Teicoplanin	2.6 \pm 0.2 (4) ^a	7.6 \pm 0.5 (5) ^a	4.3 \pm 1.6 (7) ^a	7.8 \pm 0.8 (5)	5.8 \pm 1.9 (6)

^a The *P* value versus results from control rabbits was < 0.05 by Scheffe's test after ANOVA.

between these peptides and the glycopeptides located within endocarditis vegetations may explain these findings.

Decreased susceptibility of *S. aureus* strains to vancomycin may be related to a change in the bacterial target. Studies have documented thickening of the bacterial cell wall that traps the vancomycin molecules (5, 8, 9, 17). However, these findings were obtained in vitro. To date, there are no in vivo data on cell wall thickness and structure of bacteria located within sites of infection.

In conclusion, for patients with severe infections requiring immediately effective antibiotic treatment, the possibility that glycopeptides may have limited activity should be borne in mind when selecting antistaphylococcal agents. Given the variability in the in vivo activities of glycopeptides, even against strains with in vitro susceptibility, the place for new antistaphylococcal agents, or new combinations of antistaphylococcal agents, with proven early and consistent efficacy needs to be determined.

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