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ORIGINAL ARTICLE

Investigation of the bactericidal effects of vancomycin and quinupristin/dalfopristin on *Staphylococcus aureus* isolates

Mine HOŞGÖR-LİMONCU, Şafak ERMERTCAN, Güner COŞAR
Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Ege University, 35100, Bornova, İzmir - Turkey

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Abstract: The present study aimed to determine the correlation between the bactericidal activity of vancomycin and quinupristin/dalfopristin (Q/D) on *Staphylococcus aureus* isolates and their minimal inhibition concentrations. The in-vitro susceptibilities of the 99 *S. aureus* isolates to vancomycin and Q/D were investigated by agar dilution. Thirty methicillin-resistant *S. aureus* (MRSA) and 30 methicillin-susceptible *S. aureus* (MSSA) vancomycin and Q/D susceptible isolates were involved in time-kill studies. While both MRSA and MSSA isolates were susceptible to vancomycin, 96% of both isolates were determined as susceptible to Q/D. In the time-kill test, after 6 h of incubation vancomycin exhibited a bactericidal activity of 90% on MRSA and 100% on MSSA isolates. On the other hand, in the same incubation period Q/D was 47% and 93% bactericidal for MRSA and MSSA isolates, respectively. After 24 h of incubation, while vancomycin was bactericidal for all MRSA and MSSA isolates, Q/D exhibited a bactericidal activity of 93% on MRSA isolates and 97% on MSSA isolates.

Key Words: *Staphylococcus aureus*, vancomycin, quinupristin/dalfopristin, bactericidal effect

Introduction

Over the last 2 decades, the increasing incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) has caused significant clinical problems worldwide. Moreover, MRSA isolates are often resistant not only to beta-lactam agents but also to fluoroquinolones, chloramphenicol, clindamycin, tetracyclines, and aminoglycosides (1,2). Glycopeptides have been used successfully in the treatment of serious MRSA infections for the past 30 years (3). Vancomycin, which is a glycopeptide antibiotic, is a bactericidal agent inhibiting bacterial cell wall synthesis (4). Clinical isolates of *S. aureus* that demonstrate reduced susceptibility to vancomycin have recently been described from geographically diverse sources (5). Hiramatsu et al. (6) reported the first clinical isolation of *S. aureus* with reduced susceptibility to vancomycin. Subsequently, similar organisms with reduced susceptibility to glycopeptides were reported in Michigan and New Jersey (7).

There have been a few drugs of choice for treating MRSA infections. The other treatment alternatives such as quinupristin/dalfopristin (Q/D) have become important (5).

Q/D is a new parenteral streptogramin combination that exhibits particular antibacterial potency against Gram-positive pathogens, including MRSA isolates (8-10).

Nearly all *S. aureus* isolates are inhibited by Q/D, but the bactericidal activity of this drug is much more variable (11). Bactericidal activity is important in the treatment of certain infections, particularly nosocomial infections (9).

The aim of the present study was to determine the correlation between the bactericidal activity of vancomycin and Q/D and their minimal inhibition concentrations (MIC).

Materials and Methods

Forty-nine MRSA (20 endotracheal aspirate (ETA), 8 catheter, 6 blood, 5 abscess, 5 wound, 2 sputum, 2 nasal, and 1 cerebro-spinal fluid) and 50 methicillin-susceptible

S. aureus (MSSA) (15 wound, 14 abscess, 8 ETA, 5 nasal, 4 ear, 3 catheter, and 1 sputum) strains isolated from patients treated at Celal Bayar University Hospital were used in this study. Vancomycin and Q/D were provided by Lily Inc., and Aventis Inc., respectively. The methicillin resistance of the isolates was determined (with 1 µg oxacillin disc (Oxoid)) and their in vitro susceptibilities to vancomycin and Q/D were investigated by agar dilution according to the National Committee for Clinical Laboratory Standards (NCCLS). The susceptibility breakpoint is ≤ 4 µg/ml for vancomycin and ≤ 1 µg/ml for Q/D in the NCCLS. *S. aureus* ATCC 29213 was used as a control strain (12,13).

Thirty MRSA and 30 MSSA vancomycin and Q/D susceptible isolates randomly selected were included in the time-kill studies. The time-kill tests were performed with vancomycin and Q/D according to the principles outlined by the NCCLS (14). They were performed in glass tubes containing a total volume of 2 ml. The drug concentrations were 20 µg/ml for vancomycin and 10 µg/ml for Q/D. For both drugs, these concentrations were 5-40- fold of their values of MICs for the organisms tested. The initial inoculum was targeted to be 1.5×10^6 CFU/ml. Colony counts on the control suspension (without antibiotics) were performed at time zero and on the controls and both of the test suspensions, which contained vancomycin and Q/D, at 6 and 24 h. A drug was considered bactericidal if it produced a 3-$\log_{10}$ reduction in colony counts during this incubation period (>99.9% killing). Fisher's exact test was used for statistical comparisons.

Results

MIC results of vancomycin and Q/D of 49 MRSA and 50 MSSA isolates were determined by agar dilution. The vancomycin MIC range of the isolates was 0.5-4 µg/ml, MIC_{50} values of the isolates were 1 µg/ml, and MIC_{90} values for MRSA and MSSA isolates were 1 and 2 µg/ml, respectively. The Q/D MIC range of the isolates was 0.12-2 µg/ml, MIC_{50} values for MRSA and MSSA isolates were 1 and 0.5 µg/ml, and MIC_{90} values were 1 and 0.5 µg/ml, respectively. While all of the isolates were susceptible to vancomycin, 96% of both MRSA and MSSA isolates were susceptible to Q/D. The results are shown in Table 1.

In the time-kill tests, colony counts of MRSA and MSSA isolates were determined after 0, 6 and 24 h of exposure to vancomycin and Q/D (Tables 2 and 3). After 6 h of incubation vancomycin exhibited a bactericidal activity of 90% and 100% on MRSA and MSSA isolates, respectively, whereas, for the same incubation period, the bactericidal effect of Q/D was 47% on MRSA and 93% on MSSA isolates. While, after 24 h of incubation, vancomycin was bactericidal for all MRSA and MSSA isolates, Q/D exhibited a bactericidal activity of 93% and 97% on MRSA and MSSA isolates, respectively (Table 4).

Discussion

In recent years, Gram-positive cocci have emerged as important pathogens in nosocomial infections. Currently, the therapeutic options for infections caused by resistant Gram-positive cocci are limited. The susceptibility of

Table 1. Susceptibility profiles of 99 clinical *S. aureus* isolates to vancomycin and quinupristin/dalfopristin.

	MIC (µg/ml)			% of susceptibility*
	Range	50%	90%	
Vancomycin				
MRSA (49)	0.5-4	1	1	100
MSSA (50)	0.5-4	1	2	100
Quinupristin/dalfopristin				
MRSA (49)	0.12-2	1	1	96
MSSA (50)	0.12-2	0.5	0.5	96

* Susceptible to vancomycin at ≤ 4 µg/ml and quinupristin/dalfopristin at ≤ 1 µg/ml

Table 2. Colony counts (CFU/ml) of MRSA isolates after 0, 6 and 24 h of exposure to vancomycin and quinupristin/dalfopristin.

CFU/ml	0 h	6 h			24 h		
		Control	VA*	Q/D**	Control	VA*	Q/D**
10 ↓				10		53	23
11-100	3		77	13		47	37
101-1000	43		20	27			33
1001-10,000	47	3	3	40			7
10,001-100,000	7	10		10			
100,000↑		87			100		

*VA: Vancomycin
** Q/D: Quinupristin/Dalfopristin

Table 3. Colony counts (CFU/ml) of MSSA isolates after 0, 6 and 24 h of exposure to vancomycin and quinupristin/dalfopristin.

CFU/ml	0 h	6 h			24 h		
		Control	VA*	Q/D**	Control	VA*	Q/D**
10 ↓			7	50		80	63
11-100			37	20		20	30
101-1000	23		56	23			7
1001-10,000	53			7			
10,001-100,000	24						
100,000↑		100			100		

*VA: Vancomycin
** Q/D: Quinupristin/Dalfopristin

Table 4. Bactericidal rate of vancomycin and quinupristin/dalfopristin on *S. aureus* strains after 6 and 24 h of exposure.

	Vancomycin			Quinupristin/Dalfopristin		
	Number	%		Number	%	
After 6 h						
MRSA (30)	27	90	*P = 0.11	14	47	*P = 0.000
MSSA (30)	30	100		28	93	
After 24 h						
MRSA (30)	30	100	*P = 0.24	28	93	*P = 0.5
MSSA (30)	30	100		29	97	

* Fisher's exact test

staphylococci to vancomycin and other glycopeptides appears to be waning. In recent studies it has been reported that there are staphylococci with intermediate susceptibility to vancomycin. This has ancouraged a search for new ways of treating staphylococcal infections in recent years. Q/D is active against both glycopeptide-susceptible and glycopeptide-resistant MRSA isolates (6-8).

It is important to obtain bactericidal activity in the treatment of some infections, for example, endocarditis and meningitidis. While vancomycin has a bactericidal effect on staphylococci, the bactericidal effect of Q/D varies (9,11).

In this study, all of the MRSA and MSSA isolates were susceptible to vancomycin, and 96% of both isolates were susceptible to Q/D.

In time-kill tests, in MSSA isolates the bactericidal activity of Q/D at 6 and 24 h was relatively high and closer to that of vancomycin. However, the bactericidal activity of Q/D in MRSA isolates after 6 h of incubation was distinctively low (47%) when compared to vancomycin (90%). After 24 h of incubation, the bactericidal activity of Q/D increased to 93% and became closer to the percentage of vancomycin (100%).

In their study with 516 *S. aureus* isolates, Fuchs et al. (11) determined the MIC range of Q/D to be 0.12-2 µg/ml in 199 MRSA isolates with MIC_{50} and MIC_{90} values to be 1 µg/ml, and the susceptibility rate to be 94%. In 317 MSSA isolates they determined the MIC range to be 0.12-2 µg/ml, MIC_{50} and MIC_{90} values to be 0.5 µg/ml and the susceptibility rate to be 99%. They conducted a comparative study of the bactericidal activity of Q/D and vancomycin on 39 *S. aureus* isolates and found that Q/D and vancomycin were 56% and 64% bactericidal, respectively. The bactericidal activity of Q/D started 2-6 h earlier than that of vancomycin.

In a study conducted by Hoban et al. (15) using microdilution, the MIC_{90} values in 270 *S. aureus* isolates were 0.5 µg/ml. With the time-kill method they observed that the bactericidal activity of Q/D was similar to that of vancomycin.

Betriu et al. (1), in their study with 225 MRSA isolates, found that the MIC range of Q/D was 0.1-2 µg/ml, and MIC_{50} and MIC_{90} values were 0.2 and 0.5 µg/ml, respectively. The MIC range of vancomycin was determined to be 0.2-1 µg/ml, and MIC_{50} and MIC_{90} values to be 1 µg/ml.

In the study by Kang and Rybak (10), while the bactericidal activity of Q/D was more rapid than that of vancomycin in the first hours of incubation, at 24 h it was lower in the time-kill method.

In their study on 10 MLS_B resistant *S. aureus* isolates, Fuchs et al. (9) observed that vancomycin was bactericidal on 5 isolates, whereas Q/D did not show any bactericidal activity.

The study MIC values of vancomycin and Q/D were similar to MIC values obtained in the studies discussed above.

The bactericidal activity of Q/D was closer to that of vancomycin in *S. aureus* isolates in the discussed time-kill studies (10,11,15). Furthermore, in this study, the bactericidal activities of vancomycin and Q/D were similar at 24 h. There was a contradiction between the bactericidal activity of Q/D at 6 h and the results of Fuchs and Kang et al. (9,10).

In this study vancomycin was fairly effective on staphylococci. It was demonstrated that with 24 h of incubation, the bactericidal activity of Q/D on staphylococci was comparable to that of vancomycin.

The lower bactericidal activity of Q/D after 6 h of incubation showed that vancomycin will also be important in the future, especially in the management of *S. aureus* infections like meningitis and bacteremia, in which rapid bactericidal activity is necessary.

Corresponding author:
Mine HOŞGÖR-LİMONCU
Department of Pharmaceutical Microbiology,
Faculty of Pharmacy, Ege University,
35100, Bornova, İzmir - Turkey
E-mail adress: hosgorm@pharm.ege.edu.tr

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