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Synergy of Arbekacin-based Combinations Against Vancomycin Hetero-intermediate *Staphylococcus aureus*

This study was undertaken to evaluate the in vitro activities of arbekacin-based combination regimens against vancomycin hetero-intermediate *Staphylococcus aureus* (hetero-VISA). Combinations of arbekacin with vancomycin, rifampin, ampicillin-sulbactam, teicoplanin, or quinipristin-dalfopristin against seven hetero-VISA strains and two methicillin-resistant *S. aureus* strains were evaluated by the time-kill assay. The combinations of arbekacin with vancomycin, teicoplanin, or ampicillin-sulbactam showed the synergistic interaction against hetero-VISA strains. Data suggest that these arbekacin-based combination regimens may be useful candidates for treatment options of hetero-VISA infections.

Key Words : *Arbekacin; habekacin; Rifampin; Sultamicillin; Teicoplanin quinipristin-dalfopristin; Vancomycin Resistance; Staphylococcus aureus; hetero-VISA; Time-kill assay*

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INTRODUCTION

Widespread emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) since the 1980s has led to the popular use of glycopeptides in clinical practice for more than 20 yr. Since the first report of vancomycin-intermediate *S. aurues* (VISA) in Japan (1), more than 20 cases of VISA infections have been reported (2). Furthermore, three isolates of vancomycin-resistant *S. aureus* (VRSA) (minimal inhibitory concentration [MIC] $\geq$32 mg/L) which had been reported since 2002 from the United States added more serious concern (3-5). Another category of decreased susceptibility to glycopeptide is heterogeneous resistance to vancomycin (hetero-VISA). Isolates of hetero-VISA have been reported from various parts of the world (6-10). Although clinical relevance of hetero-VISA is yet determined (11-14), this could be regarded as an early stage to vancomycin resistance (2, 15). Prudent use of vancomycin as well as the development of alternative therapeutic options against MRSA is required to prevent the further emergence of vancomycin-nonsusceptible *S. aureus*.

Arbekacin, a derivative of the aminoglycoside dibekacin (16), has been reported to have good in vitro activity against Gram-positive bacteria including MRSA (17, 18). Previous reports showed that the majority of MRSA isolates in Europe and Japan were susceptible to arbekacin (19). Combination of arbekain and vancomycin also showed a synergistic interaction against MRSA in vitro (20). However, there have been no reports about efficacy of arbekacin-based combination regimens against *S. aureus* with reduced susceptibility to vancomycin, particularly hetero-VISA.

In this study, we investigated the in vitro activities of arbekacin-based combination regimens with vancomycin, teicoplanin, rifampin, ampicillin-sulbactam, or quinupristin-dalfopristin against hetero-VISA isolated from Korea, Japan, and India.

MATERIALS AND METHODS

Bacterial strains

Seven isolates of hetero-VISA from clinical specimens were used in this study. Four isolates were from Korea (K1272, K1299, K193, and K237), two from Japan (Mu3 and J51),

and one from India (I93). Two vancomycin-susceptible MRSA strains (MRSA120 and MRSA202) were also tested. All nine strains were resistant to oxacillin. Reduced susceptibility to vancomycin of *S. aureus* isolates was confirmed by the method of population analysis as previously described (1). Hetero-VISA was defined as a strain that contained subpopulations of cells that grew on the 4 mg/L vancomycin plate at a frequency of 10^{-6} or higher (1).

Antimicrobial agents used in this study

Six antimicrobial agents were used in the in vitro susceptibility test and time-kill assay ; arbekacin (Meiji-Seika Co. Ltd., Tokyo, Japan), vancomycin (Sigma, St. Louis, MO., U.S.A.), rifampin (Sigma), ampicillin-sulbactam (Pfizer Pharmaceuticals Korea, Ltd., Seoul, Korea), teicoplanin (Sigma), and quinupristin-dalfopristin (Rhone-Poulenc Rorer, PE, U.K.).

Determination of MIC and MBC

MICs were determined by broth microdilution method of the National Committee for Clinical Laboratory Standards (NCCLS) (21). MIC determinations were performed using cation-adjusted Muller-Hinton broth (CAMHB). The minimal bactericidal concentrations (MBCs) were determined by subculture of wells with no visible growth after MIC determination. From each microtiter wells, 0.1 mL aliquots were cultured on blood agar plates (Becton-Dickinson, Sparks, MD, U.S.A.) and colonies were counted after 18-24 hr incubation at 37°C. The MBC was defined as the lowest concentrations of antibiotics that reduced the inoculums by ≥ 99.9% (21). All assays were performed in duplicate.

Time-kill assay

Time-kill assay was performed with the modified method of Watanabe et al. (14). For time-kill assay, antimicrobial agents were used at concentrations of 0.5× and 1× MIC. Time-kill assay was performed in CAMHB with isolates of 1.5×10^6 colony-forming unit (CFU)/mL. A 0.1 mL suspension of each isolates was added to 5 mL of CAMHB with each antibiotics. Bacterial culture tubes were incubated at 37°C with constant shaking for 24 hr. Arbekacin and other antimicrobial agents were tested alone, or in combination, at concentrations of 0.5× and 1× MIC. Teicoplanin and quinupristin-dalfopristin were not tested against 2 MRSA strains. Aliquots (0.1 mL) of bacterial culture were removed from cultures at 0, 4, 8, and 24 hr. Each aliquot was serially diluted in sterile saline and plated on to blood agar plates; colonies were counted on plates yielding 10-100 colonies after incubation at 35°C for 24 hr. The minimum detection limit when plating 0.1 mL of bacterial culture is about 2 $\log_{10}$ CFU/mL. Tests were performed in duplicate; results are expressed as mean $\log_{10}$ CFU/ mL. Synergy and additivity/ indifference were defined, respectively, as a ≥ 2 $\log_{10}$ CFU/ mL decrease and a <2 $\log_{10}$ CFU/mL change in the average of viable count at 24 hr for organisms treated with the combination, in comparison with the most active single drug. Antagonism is a negative interaction; the combined effect of the drug being examined is significantly less than their independent effect (22). The killing activities of various antibiotic regimens were expressed as $\log_{10}$ CFU/mL changes in the number of surviving bacteria after incubation for 0, 4, 8, and 24 hr. Serial dilution of plated samples coupled with filtration using a 0.45 micron filter was performed to minimize antimicrobial carryover effect (23).

Statistical analysis

Mean bacterial concentrations in each regimen were compared by one-way analysis of variance with the post-hoc test for multiple comparisons (SPSS, release 11.0; SPSS Inc., Chicago, IL, U.S.A.). A p-value of <0.05 was considered significant.

RESULTS

MICs and MBCs

The MICs and MBCs of arbekacin, vancomycin, rifampin,

Table 1. MICs and MBCs of antibiotics for hetero-VISA and vancomycin-susceptible MRSA strains

Antibiotics	MIC (MBC) (mg/L)								
	Isolates of hetero-VISA							Isolates of MRSA	
	Mu3	K1272	K1299	K193	K237	J51	I93	MRSA120	MRSA202
Arbekacin	4 (16)	4 (32)	4 (16)	0.5 (4)	0.25 (1)	0.25 (1)	2 (8)	2 (4)	2 (8)
Vancomycin	2 (8)	1 (8)	1 (8)	2 (8)	2 (8)	2 (8)	2 (8)	0.5 (1)	1 (2)
Rifampin	0.125 (0.25)	0.06 (0.125)	0.125 (0.12)	0.06 (0.125)	0.06 (0.125)	0.125 (0.25)	0.06 (0.125)	0.06 (0.06)	0.06 (0.06)
A/S	8/4 (16/8)	4/2 (4/2)	4/2 (4/2)	8/4 (8/4)	8/4 (16/8)	4/2 (16/8)	1/0.5 (1/0.5)	4/2 (8/4)	4/2 (16/8)
Teicoplanin	2 (4)	2 (4)	2 (8)	8 (16)	4 (8)	8 (16)	2 (8)	ND	ND
QDA	0.5 (1)	0.5 (0.5)	0.5 (1)	1 (2)	1 (2)	1 (2)	0.5 (1)	ND	ND

A/S, ampicillin-sulbactam (2:1); QDA, quinupristin-dalfopristin; ND, not done.

ampicillin-sulbactam, teicoplanin, and quinupristin-dalfopristin for nine stains are represented in Table 1. All nine strains were oxacillin-resistant and their *mecA* genes were confirmed by PCR method (data not shown). The MICs and MBCs of arbekacin ranged from 0.25 to 4 mg/L and from 1 to 32 mg/L, respectively. The MIC : MBC ratios of arbekacin ranged from 2 to 8, indicating no antimicrobial tolerance. No tolerant strains for the other antimicrobials were found.

Time-kill assays

Single regimen of arbekacin resulted in re-growth of 4 hetero-VISA (K193, Mu3, J51, and I93) and 2 MRSA strains after 8 hr. The combination regimens of arbekacin with vancomycin, ampicillin-sulbactam, or teicoplanin were synergistic against strains of MRSA and hetero-VISA either at both concentrations (0.5× and 1× MIC) or at 1× MIC (Table 2). Combination of arbekacin and vancomycin showed the synergistic killing effect in all strains of hetero-VISA and MRSA except one (MRSA120). Combination of arbekacin and rifampin showed the synergistic killing effect in only three hetero-VISA (K1272, K1299, and Mu3) strains and one MRSA strain (MRSA120). Combination of arbekacin and teicoplanin or ampicillin-sulbactam was synergistic against 4 strains out of 7 hetero-VISA strains. The combination of arbekacin and quinupristin-dalfopristin was not synergistic against all 7 hetero-VISA. Fig. 1 showed the bacterial killing effect of arbekacin based combination regimens at 0.5× and 1× MIC concentrations against a representative hetero-VISA strain, Mu3. Antagonistic interaction was not observed in any combination regimens. The combination of arbekacin and ampicillin-sulbactam was the most effective significantly at 1× MIC concentration against 5 strains out of 7 hetero-VISA strains ($p<0.05$). At 0.5× MIC concentration, however, the combination of arbekacin and vancomycin was the most effective significantly against 4 hetero-VISA strains ($p<0.05$) (Table 2).

DISCUSSION

Data from this study suggest that arbekacin-based combination regimens could be an alternative option for glycopeptides in the treatment of MRSA or hetero-VISA infections. Although clinical implications of hetero-VISA are still controversial, some reports documented the clinical failures of vancomycin treatment in patients infected by these strains (11, 12, 24). To treat infections caused by vancomycin non-susceptible *S. aureus*, some of the current antibiotics are still effective including rifampin, tetracycline, minocycline, chloramphenicol, trimethoprim-sulfamethoxazole, linezolid or quinupristin-dalfopristin (25, 26). Arbekacin has been used for the treatment of MRSA infections since 1990 in Japan (17, 18, 27). Combination of arbekacin and ampicillin-sulbactam is one of the popular regimens in the treatment of MRSA infections in Japan (28). Although arbekacin showed relatively good in vitro activity against MRSA and hetero-VISA, the administration of a single arbekacin of 0.5× or 1× MIC concentrations seems not effective due to bacterial regrowth after 8 hr in this study. As MIC:MBC ratios of arbe-

Table 2. In vitro activity of arbekacin-based combinations against 7 hetero-VISA and 2 MRSA strains*

Strain	Reduction in bacterial counts (log_{10} CFU/mL±SD) compared with the two antibiotics used alone									
	VAN+ABK		RFP+ABK		A/S+ABK		TEI+ABK		QDA+ABK	
	0.5×MIC	1×MIC	0.5×MIC	1×MIC	0.5×MIC	1×MIC	0.5×MIC	1×MIC	0.5×MIC	1×MIC
Mu3	**-4.6±0.2 S**	-2.7±0.3 S	-0.4±0.07 A	-2.2±0.08 S	-2.2±0.01 S	**-3.8±0.8 S**	-1.0±0.08 A	-2.5±0.4 S	-0.0±0.04 A	-0.4±0.7 A
K1272	-1.3±0.02 A	-2.1±0.01 S	-0.6±0.03 A	-2.1±0.07 S	-0.4±0.07 A	-0.0±0.6 A	-0.7±0.05 A	-2.3±0.03 S	-1.3±0.3 A	-0.1±0.08 A
K1299	-2.0±0 S	**-2.5±0.05 S**	**-2.5±0.4 S**	-1.3±0 A	+0.9±0.4 A	-1.1±0.08 A	+1.0±0.03 A	-2.1±0 S	+0.7±0.08 A	+0.6±0.04 A
K193	-0.2±0.01 A	**-4.3±0.1 S**	-0.3±0.08 A	-0.5±0.8 A	+0.2±0.04 A	**-4.4±0.05 S**	-1.4±0 A	-3.0±0.07 S	-0.6±0.04 A	-0.0±0.6 A
K237	**-3.9±0 S**	-1.7±0.09 A	-0.2±0.07 A	-0.6±0.04 A	+0.1±0.7 A	**-3.7±0.1 S**	-3.5±0.9 S	-2.4±0 S	-0.3±0.6 A	-0.4±0.06 A
J51	**-5.5±0.09 S**	-3.3±0.08 S	-0.4±0.6 A	-0.6±0.1 A	-0.4±1.05 A	**-4.2±0.3 S**	-0.4±0.07 A	-0.5±0.05 A	-0.0±0.1 A	+0.4±0.5 A
I93	**-5.2±0.1 S**	-2.2±0 S	-1.0±0.2 A	-0.3±0.03 A	-1.0±0.09 A	**-4.6±0.07 S**	+0.1±0.4 A	-0.0±0.09 A	+0.1±0.9 A	-0.8±0.1 A
MRSA120	-0.5±0.07 A	-0.0±0.2 A	-2.4±0.01 S	-2.2±0.01 S	-4.6±0.05 S	**-2.4±0.01 S**	ND -	ND -	ND -	ND -
MRSA202	-0.6±0.06 A	-2.1±0.02 S	-0.6±0.5 A	-0.0±0.02 A	-0.9±0.01 A	**-5.7±0.5 S**	ND -	ND -	ND -	ND -

*The combination regimen that was the most significantly effective against each strain was represented as **bold** ($p<0.05$). ABK, arbekacin; VAN, vancomycin; RFP, rifampin; A/S, ampicillin-sulbactam; TEI, teicoplanin; QDA, quinupristin-dalfopristin. S, synergic; A, additive/indifferent; ND, not done.

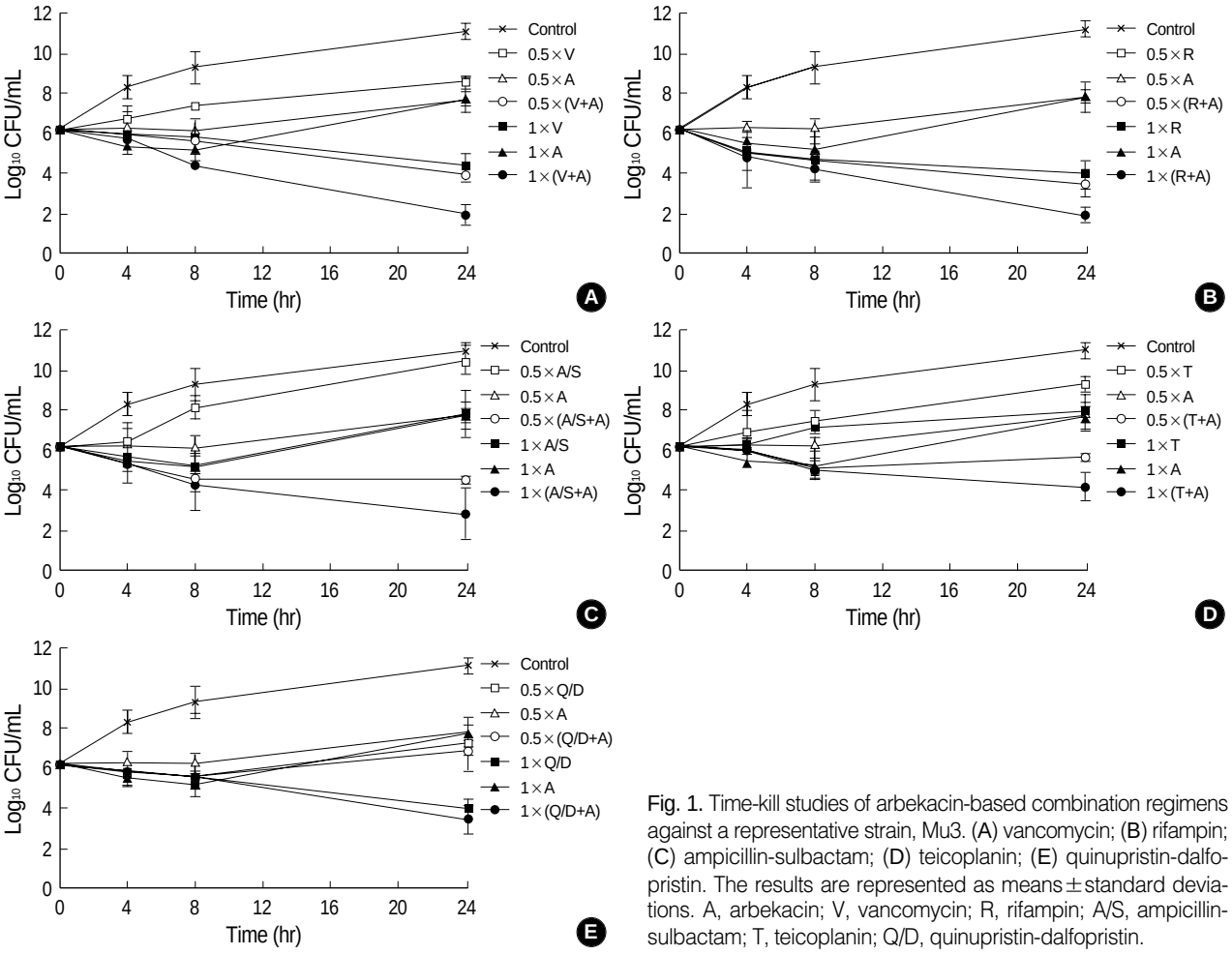

Fig. 1. Time-kill studies of arbekacin-based combination regimens against a representative strain, Mu3. (A) vancomycin; (B) rifampin; (C) ampicillin-sulbactam; (D) teicoplanin; (E) quinupristin-dalfopristin. The results are represented as means±standard deviations. A, arbekacin; V, vancomycin; R, rifampin; A/S, ampicillin-sulbactam; T, teicoplanin; Q/D, quinupristin-dalfopristin.

kacin for all strains were low, such re-growth seems not to due to antimicrobial tolerance.

In this study, combination regimens of arbekacin with vancomycin, teicoplanin, or ampicillin-sulbactam were synergistic against hetero-VISA and MRSA strains. In vitro efficacy of the combination of arbekacin and vancomycin against MRSA isolates in this study is consistent with previous data which showed the in vitro activity of combination regimens of arbekacin and vancomycin or daptomycin against MRSA and hetero-VISA strains (14, 20, 29, 30). Particularly, synergistic interaction of the combination of arbekacin and ampicillin-sulbactam against MRSA and hetero-VISA in the time-kill assay could provide the rationale of clinical uses of this combination in the treatment of MRSA infections in Japan.

This study has some limitations. First, arbekacin concentrations used in this study (0.125-4 mg/L) was lower than the maximally achievable concentration in healthy adults after 100 mg of arbekacin by one-hour intravenous infusion (7.56 mg/L, range 5.6-10 mg/L) (31). However, since strains used in the study showed relatively low MIC (0.25-4 mg/L) of arbekacin, 0.5× or 1× MIC concentration could not simulate the actual situation in the human body. This low concentration of arbekacin could affect the in vitro killing efficacy of the drug shown in the study. Second, data from the in vitro study may not reflect the in vivo drug efficacy because in vitro model could not reflect the pharmacodynamic features of antibiotics. We are now developing the experimental infection model by MRSA and hetero-VISA to evaluate the in vivo efficacy of arbekacin.

In summary, in vitro data could suggest the possibility of an alternative option in the treatment of MRSA infections which could circumvent the selective pressure of glycopeptides in the clinical practice.

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