Efficacy of ME1036 against methicillin-resistant *Staphylococcus aureus* and vancomycin-insensitive *S. aureus* in a model of hematogenous pulmonary infection

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Running title: Efficacy of ME1036 in hematogenous lung infection

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ABSTRACT

ME1036, a novel parenteral carbapenem, was developed for the treatment of MRSA and vancomycin-intermediate S. aureus (VISA).

A model of hematogenous pulmonary infection was induced in mice by tail vein injection of MRSA strain NUMR10 or VISA Mu50 enmeshed in agar beads. Twenty-four h after infection, mice were treated twice daily for 7 days with 20 mg/kg/day vancomycin or ME1036. Mice infected with VISA were also pretreated with cyclophosphamide to induce an immunocompromised state. Twelve h after the final drug treatment, the number of viable bacteria in the lungs was counted.

VCM decreased the number of viable MRSA in the lungs in comparison with the control, although the difference was not significant ($\log_{10}$ cfu/lung [mean±SEM] = 6.876±0.54 and 8.25±0.41, respectively). In contrast, treatment with ME1036 resulted in a significant decrease ($\log_{10}$ cfu/lung= 2.69±0.44 [n=6]; $P<0.0001$) in the number of viable MRSA compared with both the VCM-treated and control mice. In the VISA-infected mice, ME1036 significantly reduced the number of viable bacteria compared to VCM and control ($\log_{10}$ cfu/lung = 3.65±0.68 for ME1036 vs. 5.71±0.75 for VCM [$P<0.05$] and 7.07±0.45 for control [$P<0.001$]).

ME1036 produced >3 log10 reduction versus control against both MRSA strains: >5 log for the vancomycin-susceptible and 3.4 log for the VISA, whereas vancomycin produced <1.3 log for both strains.

Key words: ME1036, pulmonary infection, MRSA, vancomycin-intermediate, Staphylococcus aureus

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first identified in the 1960s, and the acquisition by MSSA of genes encoding an additional penicillin-binding protein, PBP-2a, which has a low affinity for β-lactams, resulted in the emergence of MRSA in many health care institutions around the world. Currently, glycopeptides such as vancomycin (VCM) and teicoplanin still provide effective therapy against most strains of MRSA. However, the first MRSA to acquire resistance to VCM was isolated from a Japanese patient in 1996 [1]. Subsequent isolation of several VCM-intermediate *S. aureus* (VISA) strains from USA, France, Korea, South Africa, and Brazil has confirmed that emergence of VCM resistance in *S. aureus* is a global issue [2]. Subsequently, new agents against MRSA and VISA were developed, including linezolid, daptomycin, and quinupristin-dalfopristin. ME1036, a novel parenteral carbapenem, was developed to combat MRSA or VISA [3-4], and it is expected to be useful for the treatment of severe staphylococcal infections such as pneumonia, bacteremia, and pulmonary abscesses.

To evaluate the efficacy of antibacterial agents and the pathogenesis of blood-borne staphylococcal pneumonia, we previously established a murine model of pulmonary infection, wherein infection is induced by the intravenous injection of agar-enmeshed *S. aureus* [5-8]. The aims of the present study were to compare the activity of ME1036 with VCM against pulmonary infection caused by MRSA and VISA and to analyze the antibacterial and histopathological effects and the pharmacokinetics of ME1036.

MATERIALS AND METHODS

Laboratory animals

Six-week-old male ddY specific pathogen-free mice (25–30 g body weight) were purchased from Shizuoka Agricultural Cooperative Association Laboratory Animals (Shizuoka, Japan).

All animals were housed at the Laboratory Animal Centre for Biomedical Science at Nagasaki University in a pathogen-free environment and received sterile food and water. All experimental protocols were approved by the Ethics Review Committee for Animal Experimentation at our institution.

Bacterial strain

Two strains of *S. aureus* were examined. Strain NUMR101 was isolated at Nagasaki University Hospital from the blood of an inpatient with pneumonia. Mu50, a VISA strain, was kindly provided by K. Hiramatsu (Juntendo University, Tokyo, Japan). The bacteria were stored at −70°C in brain-heart infusion broth (BBL Microbiology System, Cockeysville, MD) supplemented with 10% (v/v) glycerol and 5% (w/v) skim milk (Yukijirushi Co., Tokyo, Japan) until use.

Determination of minimum inhibitory concentrations (MICs)

ME1036 (Meiji Pharmaceutical Co., Tokyo, Japan) was dissolved in 0.01 N NaOH, 2% glucose, and 1 mM phosphate-buffered saline. VCM (Shionogi Pharmaceutical Co., Osaka, Japan) was dissolved in sterile water immediately before use. The MIC of each agent was determined by the microplate dilution technique using Muller-Hinton medium, with an inoculum size of 5×10^5 colony forming units (cfu)/ml. The MIC was defined as the lowest concentration of the test agent that inhibited visible growth of bacteria after 18 h at 37°C. The MICs of ME1036 and VCM for NUMR101 were both 1.0 μg/ml, and those for Mu50 were 2.0 and 8.0 μg/ml, respectively.

Mouse model of hematogenous pneumonia

The mouse model of hematogenous pneumonia and the method of inoculation were previously described [5-8]. Briefly, *S. aureus* was cultured on trypticase soy agar (BBL

Microbiology System, Cockeysville, MD)-based sheep blood agar plates for 24 h at 37°C. Bacteria were suspended in endotoxin-free sterile saline and harvested by centrifugation (3000 × g; 4°C; 10 min). The organisms were resuspended in cold sterile saline and diluted to 2×10^9 to 4×10^9 cfu/ml, as estimated by turbidimetry. The numbers of bacteria were verified by inoculating duplicates of serial dilutions onto blood agar plates and counting the cfu after 48 h at 37°C. The suspension was warmed to 45°C, and 10 ml of the suspension was mixed with 10 ml of 4% (w/v) molten Noble agar (Difco Laboratories Detroit, MI) at 45°C. The agar-bacterium suspension (1.0 ml) was placed in a 1.0-ml syringe, and the suspension was rapidly injected through a 26-gauge needle into 49 ml of rapidly stirred ice-cooled sterile saline. This resulted in solidification of the agar droplets into beads approximately 200 μm in diameter. The final concentration of agar was 0.04% (w/v), and the final number of bacteria was 2×10^7 to 4×10^7 cfu/ml. Mice infected with VISA were pretreated with cyclophosphamide at Day -3 and at Day -1 before inoculation to induce an immunocompromised state. Mice were injected in the tail vein with 0.20 to 0.25 ml (10 ml/g of body weight) of the suspension of bacteria enmeshed in agar beads at Day 0. Treatment started at day 1 and ended at day 7.

Treatments

Mice infected with MRSA were treated every day for 7 days by intraperitoneal injection of ME1036 (200 mg/kg/day, bid; n=6), VCM (200 mg/kg/day, bid; n=6), or saline (control; n=6). Mice infected with VISA were treated every day for 10 days by intraperitoneal injection of ME1036 (100 mg/kg/day, bid; n=6), VCM (100 mg/kg/day, bid; n=6), or saline (control; n=6). Twelve h after the final drug treatment, mice were sacrificed and the number of viable bacteria in the lungs was counted.

Bacteriological and histopathologica, analyses

Each group of animals was sacrificed by cervical dislocation at twelve h after the final drug

treatment. After exsanguination, the lungs were dissected and removed under aseptic conditions. Organs used for bacteriological analyses were homogenized and cultured quantitatively by serial dilutions on blood agar plates. Lung tissue for histological examination was fixed in 10% buffered formalin and stained with hematoxylin-eosin. The histological analysis only performed in the VISA-infected animals.

Measurement of the lung and serum concentrations of drugs

Mice (n=4 per group) were sacrificed by cervical dislocation 0.25, 0.5, 1, 2, 4, and 6 h after the completion of treatment. Serum was separated after the blood had clotted. The infected mice were used for the measurement of lung and serum concentrations. They were different from those for bacteriological and hitopathological analyses. There are 6 timepoints and 4 animals per group, were 24 animals (6x4) used per treatment group. The lungs were removed, washed briefly, and cryohomogenized with saline. These samples were immediately frozen and stored at $-80°C$ for a few days until the assay was performed. The concentration of ME1036 was measured by the paper disc (bioassay) method. The test organism was *Bacillus subtilis* ATCC6633. The concentrations of VCM were measured by fluorescence polarization immunoassay [9]. The pharmacokinetic parameters were calculated on the basis of non-compartment model and 1-compartment model with the software; WinNonlin(Ver.5.0, Pharsight Corporation). The PK/PD parameters were calculated with Microsoft Excel. The non-protein-bound fraction of the drug in mouse serum was 54% for ME1036 and 75% for vancomycin.

Statistical analysis

Bacteriological data were expressed as means ± SEM. Survival data were expressed by Kaplan-Meyer analysis. Differences between groups were examined for statistical significance using an unpaired *t*-test. A *P* value of less than 0.05 was considered to indicate a

statistically significant difference.

RESULTS

Therapeutic effects of antibacterial agents

Treatment with VCM decreased the number of viable MRSA in the lungs in comparison with the control without any significance ($\log_{10}$ cfu/lung [mean±SEM] = 6.876±0.54 and 8.25±0.41, respectively [n=6 per group]). In contrast, treatment with ME1036 ($\log_{10}$ cfu/lung= 2.69±0.44 [n=6]) resulted in a significant decrease ($P<0.0001$) in the number of viable MRSA compared with both the VCM-treated and control mice (Fig. 1A). In the VISA-infected mice, ME1036 significantly reduced the number of viable bacteria compared to VCM and control ($\log_{10}$ cfu/lung = 3.65±0.68 for ME1036 vs. 5.71±0.75 for VCM [$P<0.05$] and 7.07±0.45 for control [$P<0.001$] [n = 6 per group]; Fig. 1B). The figures refer to absolute colony counts.

Histopathological examination

Lung specimens of mice infected with VISA Mu50 were examined 7 days after treatment. The lungs showed abscesses consisting of a central zone of a bacterial colony with infiltrating acute inflammatory cells (Fig. 2). The ME1036-treated (Fig. 2A) and VCM-treated groups (Fig. 2B) exhibited fewer abscesses and less inflammation than the control group (Fig. 2C). Fewer abscesses were observed in the ME1036 group.

Serum and lung ME1036 and VCM concentrations

The peak concentrations of ME1036 and VCM were 78.71 and 61.03 µg/ml in plasma and 20.03 and 26.19 µg/g in lung tissues, respectively (Fig. 3). Table 1 shows the pharmacodynamic and pharmacokinetic parameters in the lung tissues of mice.

DISCUSSION

In the current studies, we used a mouse model hematogenous pulmonary infection to assess the effects of ME1036 on MRSA and VISA. This model is considered to accurately reflect the efficacy of antimicrobial agents against pulmonary infection with MRSA and VISA. Using this model, we previously showed the efficacy of linezolid (the first oxazolidinone agent), DX-619 (a new quinolone agent), and quinupristin-dalfopristin (a complex of streptogramin A and B) against MRSA and VISA, whereas VCM was consistently ineffective in this model.[5,7,8] In the present study, measurement of bacterial numbers and histopathological analyses revealed that ME1036 was more effective than VCM in the MRSA and VISA lung infection models.

When the level of protein binding is taken into account, the free-fraction areas under the concentration-time curve between 0 and infinite hours were similar for ME1036 and VCM. The data obtained from these pharmacokinetic studies, however, cannot account for the superior efficacy of ME1036 in this model.

In the previous rabbit model, the bacterial counts in the controls were in the same range. The MIC for the inoculum size (1.6×10^7 CFU/spot) was comparable to the bacterial counts within the vegetation in this model. The MIC of ME1036 at the larger inoculum size was lower than that of vancomycin. The sub-MIC effect and the MIC observed at large inoculum sizes may contribute to the better in vivo efficacy of ME1036 compared to that of vancomycin.[4].

In conclusion, we showed that ME1036, a novel parenteral carbapenem, can reduce the number of bacteria in a MRSA hematogenous infection mouse model and that it is significantly more effective than VCM at improving the survival rate of immunocompromised mice infected with VISA. Thus, it is likely that ME1036 will be clinically effective for treating MRSA and VISA infections.

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FIGURE LEGENDS

Figure 1. Effects of ME1036 and VCM on the bacterial numbers in lungs of animals with VISA hematogenous pulmonary infection. Mice were treated with each agent after infection. ME1036 produced >3 log10 reduction versus control against both MRSA strains: >5 log for the vancomycin-susceptible and 3.4 log for the VISA, whereas vancomycin produced <1.3 log for both strains.

Figure 2. Histopathological examination of lung specimens from mice sacrificed three days after treatment. (hematoxylin and eosin staining; original magnification, ×50). (A) ME1036-treated mice. (B) VCM-treated mice. (C) Control. Note that the inflammatory process is less severe in the ME1036-treated group than in the other groups.

Figure 3. Pharmacokinetics of ME1036 (100 mg/kg/day) (A) and VCM (100 mg/kg/day) (B) in the lungs of infected mice. Drugs were administered intraperitoneally twice daily starting 24 h after infection. Results are presented as means±SD.

Table 1. Selected pharmacokinetic and pharmacodynamic parameters for mean profiles for ME1036 and VCM in the serum and lung of infected mice.

	Sample	C_{max} (µg/mL or g)	$t_{1/2}$ (hr)	AUC_{0-2h} (µg· hr/mL or g)	AUC_{0-12h} (µg· hr/mL or g)	AUC_{0-inf} (µg· hr/mL or g)	T>MIC (%) MRSA	VISA	AUC_{0-24} f/MIC MRSA	VISA	C_{max}f/MIC MRSA	VISA
ME 1036	Plasma	78.71	0.21	62.04	62.24	62.24	17	15	67.2	33.6	42.5	21.3
	Lung	20.83	0.27	14.87	15.10	15.10	16	13	16.3	8.2	11.2	5.6
VCM	Plasma	61.03	0.44	62.90	67.13	67.13	23	14	100.7	12.6	45.8	5.7
	Lung	26.19	0.57	31.85	36.37	36.37	25	12	54.6	6.8	19.6	2.5

Pharmacokinetic data from MRSA-infected mice treated with each drug at 100 mg/kg once a day after bacterial inoculation. Pharmacokinetic parameters were calculated from the arithmetic means of plasma and lung concentrations (mean values for four animals).
AUC_{0-6}, area under the concentration-time curve from 0 to 6 h; AUC_{0-6}, area under the concentration-time curve from 0 to infinity h; C_{max}, maximum concentration; $t_{1/2}$, half-life.
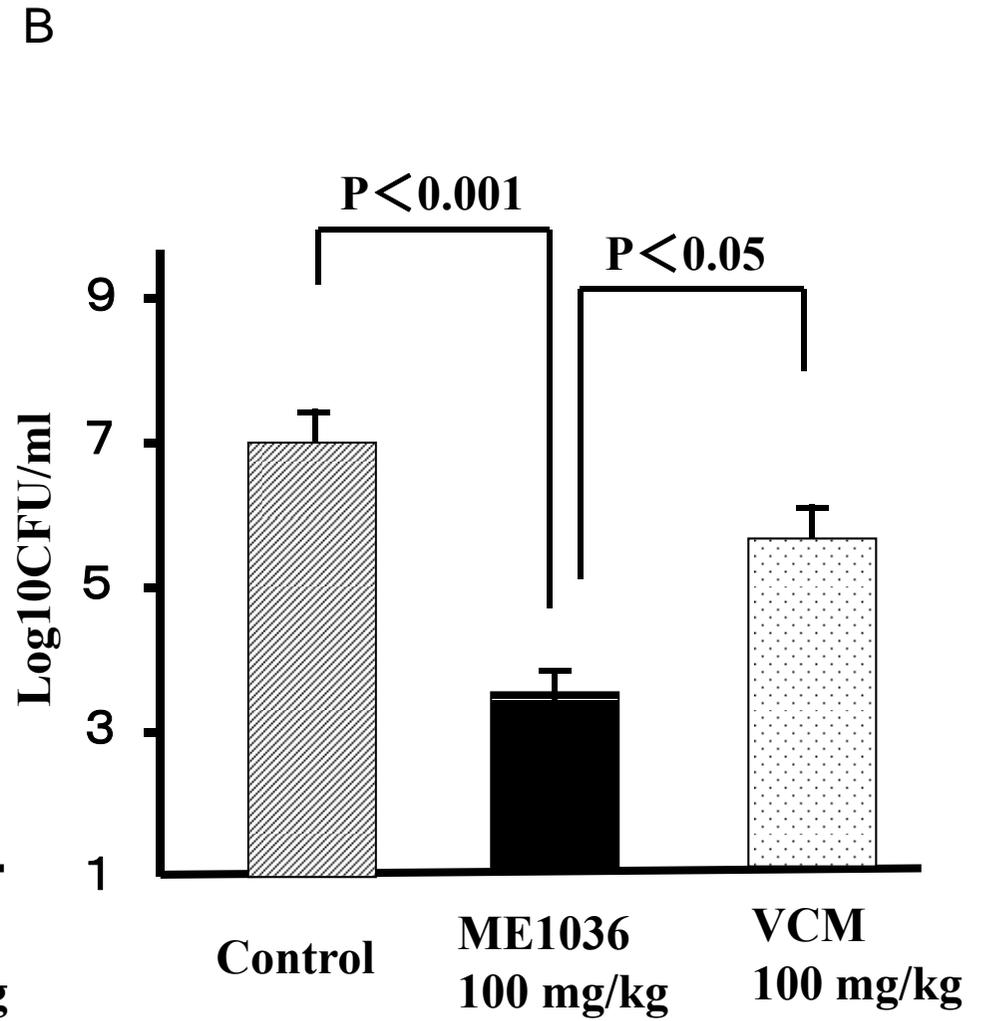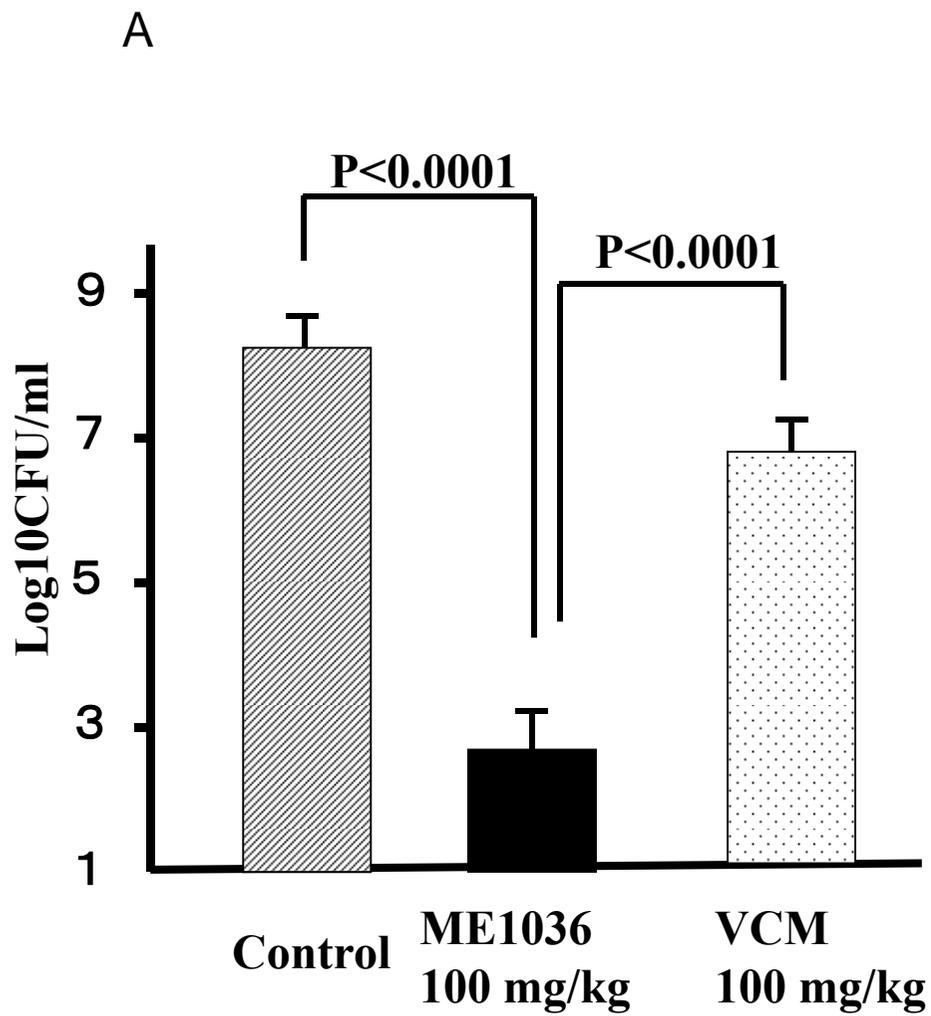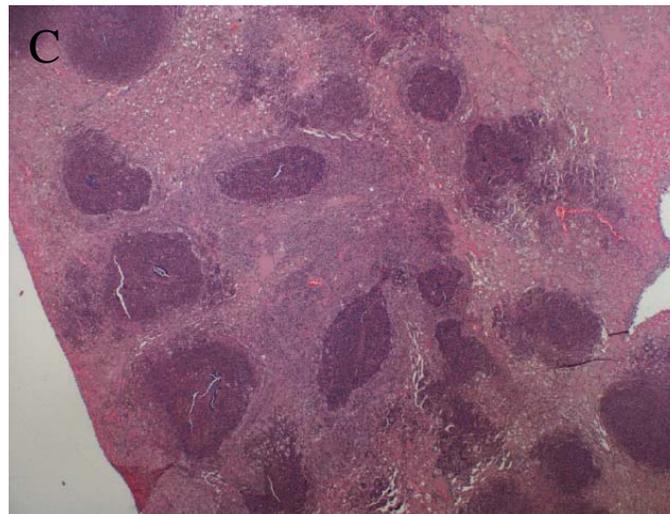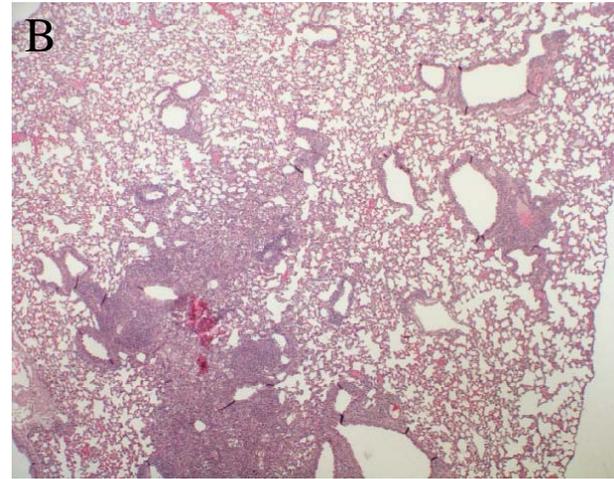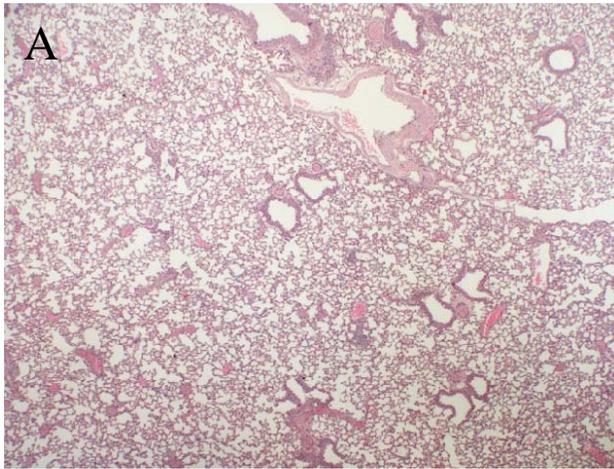
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