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Short communication

Efficacy of linezolid against Panton–Valentine leukocidin (PVL)-positive meticillin-resistant *Staphylococcus aureus* (MRSA) in a mouse model of haematogenous pulmonary infection

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ABSTRACT

Many strains of community-acquired meticillin-resistant *Staphylococcus aureus* (MRSA) have a pore-forming leukotoxin, known as Panton–Valentine leukocidin (PVL), which can cause severe necrotising pneumonia. Linezolid (LZD) is a new antibacterial agent with potent antibacterial activity against MRSA. In this study, a mouse model of haematogenous pulmonary infection was used to compare the efficacies of LZD and vancomycin (VAN) against pulmonary infection caused by PVL-positive *S. aureus*. Following antibiotic administration for 3 days, the number of viable bacteria (mean ± standard error of the mean) in the control, VAN and LZD groups was 6.77 ± 0.14, 5.29 ± 0.27 and 4.25 ± 0.33 log colony-forming units/lung, respectively. LZD significantly decreased the number of viable bacteria in the lungs compared with the control and VAN groups ($P < 0.05$). The survival rate at Day 7 post-inoculation was higher in the LZD group (100%) than in the VAN group (50%) or the control group (0%). Histopathological examination and cytokine analysis also showed the beneficial efficacy of LZD compared with VAN. In conclusion, LZD significantly reduced bacterial numbers and inflammation in a mouse model of PVL-positive *S. aureus* haematogenous infection and improved the survival rate of infected mice compared with VAN. LZD is clinically effective against PVL-positive *S. aureus*.

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1. Introduction

Community-acquired meticillin-resistant *Staphylococcus aureus* (MRSA) infection has been increasing worldwide [1]. Many strains of community-acquired MRSA have a phage harbouring the Panton–Valentine leukocidin (PVL) genes [2–4]. PVL is a pore-forming leukotoxin [5,6], and PVL-positive *S. aureus* can cause primary skin and soft-tissue infections [7] as well as severe necrotising pneumonia in young immunocompetent patients [6,7]. The mortality rate is 75% [4] and autopsy reveals extensive necrotic and haemorrhagic lesions of the trachea, bronchi, alveolar septa and parenchyma. Thus, new treatment strategies are needed for PVL-positive *S. aureus* infection.

Linezolid (LZD) has potent antibacterial activity against Gram-positive cocci, vancomycin (VAN)-resistant enterococci and MRSA. LZD has been reported to be more effective than VAN in achieving microbiological eradication for the treatment of MRSA infections [8].

Previously, we established a mouse model of pulmonary infection with *S. aureus* by intravenous (i.v.) injection of bacteria enmeshed in agar beads [9–11]. The aim of this study was to compare the activity and efficacy of LZD and VAN against PVL-positive *S. aureus* haematogenous pulmonary infection in a model mouse.

2. Materials and methods

2.1. Bacterial strains and culture conditions

PVL-positive *S. aureus* was kindly provided by Prof. T. Yamamoto (Niigata University, Niigata, Japan). The strain was classified as staphylococcal cassette chromosome *mec* (SCC*mec*) type IV [6]. The bacteria were stored at −80 °C in a Microbank® (Pro-Lab Diagnostics, Ontario, Canada) until use. Bacteria were grown at 37 °C on Mueller–Hinton II agar (Becton Dickinson and Company, Sparks, MD) or in brain–heart infusion (BHI) broth (BBL Microbiology System, Cockeysville, MD).

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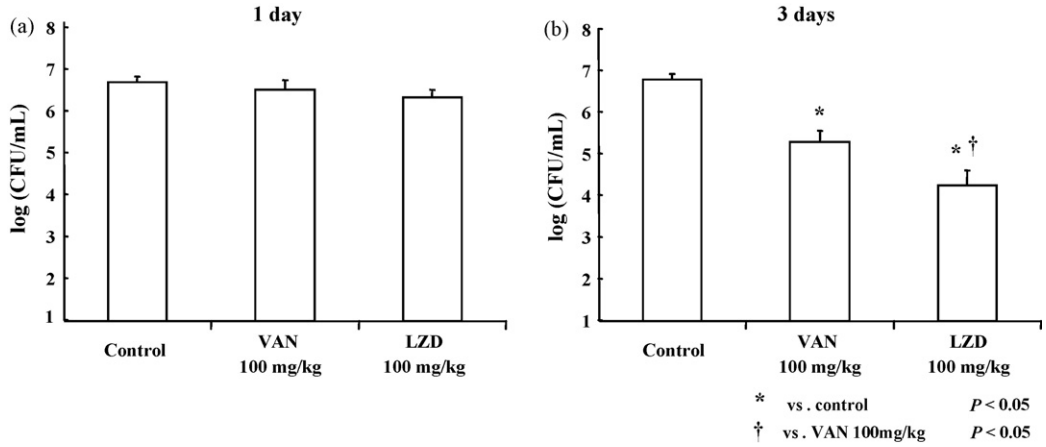

Fig. 1. Effects of linezolid (LZD) and vancomycin (VAN) on the number of bacteria in the lungs of mice with Panton–Valentine leukocidin-positive *Staphylococcus aureus* haematogenous infection treated with each agent for (a) 1 day or (b) 3 days. CFU, colony-forming units.

2.2. Antibiotics

LZD (Pfizer Japan Inc., Tokyo, Japan) and VAN (Shionogi Pharmaceutical Co., Osaka, Japan) were dissolved in sterile water immediately before use.

2.3. Determination of minimum inhibitory concentrations (MICs)

The MIC of each agent was determined by the microplate dilution technique. Mueller–Hinton II medium (Becton Dickinson and Company) and an inoculum size of 5×10^5 colony-forming units (CFU)/mL was used. The MIC was defined as the lowest concentration of test agent that inhibited visible growth of bacteria after 18 h of incubation at 37 °C.

2.4. Laboratory animals

Six-week-old, male, ddY, specific pathogen-free mice were purchased from Shizuoka Agricultural Cooperative Association Laboratory Animals (Shizuoka, Japan). All animals were housed in a pathogen-free environment and received sterile food and water in the Laboratory Animal Centre for Nagasaki University Graduate School of Biomedical Sciences.

2.5. Inoculum

Inoculation was performed as described previously [9–11]. Briefly, PVL-positive *S. aureus* was cultured overnight on Mueller–Hinton II agar plates at 37 °C. Bacteria were suspended in endotoxin-free sterile saline and harvested by centrifugation (3000 rpm, 4 °C, 10 min). Microorganisms were re-suspended in cold sterile saline and diluted to between 2×10^{10} and 4×10^{10} CFU/mL or 2×10^{11} and 4×10^{11} CFU/mL as estimated by turbidimetry. The suspension was warmed to 42 °C and 10 mL of the suspension was then mixed with 10 mL of 4% (w/v) molten noble agar (Difco Laboratories, Detroit, MI) at 42 °C. The agar–bacterium suspension (1.0 mL) was placed into a 1.0 mL syringe and the suspension was rapidly injected via a 26 gauge needle into 49 mL of rapidly stirred ice-cooled sterile saline. This procedure resulted in solidification of the agar droplets into beads of ca. 200 μm diameter. The final concentration of agar was 0.04% (w/v) and the final number of bacteria was 2×10^8 to 4×10^8 CFU/mL for cytokine, bacteriological and histopathological studies or 2×10^9 to 4×10^9 CFU/mL for survival studies.

2.6. Animal model of haematogenous pneumonia

A total of 0.25 mL of the suspension containing agar beads with bacteria was injected into the tail vein of each mouse (10 mL/kg body weight). The method used for inducing infection has been described in detail elsewhere [9–11]. Treatment commenced a day after inoculation by intraperitoneal administration. Animals were allocated into three groups: LZD (100 mg/kg/dose); VAN (100 mg/kg/dose); or control. Each drug was administered twice daily for 1 day or 3 days for the bacteriological study ($n = 10$ in each group), 3 days for histopathological and cytokine studies ($n = 10$ in each group) or 7 days for survival studies ($n = 6$ in each group).

2.7. Bacteriological, histopathological and survival examinations

Mice were sacrificed by cervical dislocation 12 h after administration of antibiotics. Following exsanguination, the lungs were dissected and removed under aseptic conditions. Organs used for bacteriological analysis were homogenised and cultured quantitatively by serial dilution on Mueller–Hinton II agar plates. Lung tissue for histological examination was fixed in 10% buffered formalin and stained with haematoxylin–eosin. Specimens were examined under a microscope and total abscesses were counted. The lung area was calculated using cross-section paper.

2.8. Enzyme-linked immunosorbent assay (ELISA)

Concentrations of tumour necrosis factor-alpha (TNFα), macrophage-inflammatory protein-2 (MIP-2) and interleukin (IL)-1β in the lung homogenates were assayed using mouse cytokine ELISA kits (R&D Systems, Minneapolis, MN).

2.9. Statistical analysis

Bacteriological and cytokine data were expressed as the mean ± standard error of the mean, and survival data were expressed by Kaplan–Meier curves. Differences between groups were examined using the unpaired *t*-test. *P*-values of <0.05 were considered statistically significant.

3. Results

3.1. Minimum inhibitory concentration of each antibiotic for Panton–Valentine leukocidin-positive Staphylococcus aureus

The MICs of LZD and VAN for PVL-positive *S. aureus* were 2 μg/mL and 1 μg/mL, respectively.

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