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Antimicrobial and immunomodulatory effects of tedizolid against methicillin-resistant *Staphylococcus aureus* in a murine model of hematogenous pulmonary infection

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ABSTRACT

Tedizolid (TZD) is a second-generation oxazolidinone and demonstrates potent in-vitro activity against multidrug-resistant Gram-positive bacteria. Phase III studies in patients with acute bacterial skin and skin structure infections (ABSSSI) have demonstrated the non-inferiority of TZD to linezolid (LZD). However, there are only a few studies that show the effect of TZD in pulmonary infections. In this study, we investigated the effect of TZD in a murine model of hematogenous pulmonary infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA). The mice were treated either twice daily with saline (control), 25 mg/kg of vancomycin (low-VAN), 110 mg/kg of vancomycin (high-VAN), 120 mg/kg of LZD or once daily with 20 mg/kg of TZD. As compared to the control, the low- and high-VAN treatment groups, LZD and TZD significantly improved the survival rate, reduced the bacterial count in the lungs. Furthermore, TZD decreased the area of central bacterial colony zone (CBCZ) at 36 h post-inoculation, compared with the control. In addition, we investigated the immunomodulatory effect of TZD by evaluating the plasma concentrations of the inflammatory cytokines. Although there were no significant differences in the bacterial count in the lungs amongst the drugs at 26 h post-inoculation, TZD and LZD significantly improved the plasma concentrations of TNF-alpha, IL-6 and MIP-2, in comparison with the control. In this study, both TZD and LZD demonstrated antimicrobial and immunomodulatory efficacy in a murine model of hematogenous pulmonary infection caused by MRSA.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first identified in the 1960s, 2 years after the initiation of the clinical use of methicillin. Since then, MRSA has spread worldwide and is a significant pathogen associated with many nosocomial and healthcare-associated infections, such as bacteremia, endocarditis, pneumonia, and skin and soft tissue infections (Liu et al., 2012). Recently, panton-valentine leukocidin (PVL)-positive community-associated (CA)-MRSA, especially the USA300 strain, is widespread in the community- and hospital settings (Grundmann et al., 2006; Holzknecht et al., 2010; Popovich et al., 2008). Thus, MRSA infections are diseases of emerging importance, which need our attention for effective treatment.

During the past decade, several anti-MRSA agents have been developed. Among these, linezolid (LZD), the first-generation oxazolidinone, has certain distinct characteristics: its mechanism of action is by inhibition of protein synthesis; its oral bioavailability is 100%; its tissue penetration, including into the epithelial lining fluid (ELF) of the lungs and the infected skin and soft tissues, is good (Liu et al., 2012; Rodvold and McConeghy, 2014). Additionally, LZD showed an immunomodulatory effect, such as inhibition of the inflammatory cytokine production (Yoshizawa et al., 2012; Sharma-Kuinkel et al., 2013; Zarogoulidis et al., 2012). In the published guidelines for the treatment of MRSA infection, LZD is one of the first-line agents for the treatment of skin and soft-tissue

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infections, pneumonia and bone and joint infections caused by MRSA (Liu et al., 2012). However, some outbreaks of LZD-resistant pathogens have been reported (Gu et al., 2013).

Tedizolid (TZD) is a second-generation oxazolidinone and has demonstrated potent in-vitro activity against multidrug-resistant Gram-positive bacteria, including some LZD-resistant strains (Locke et al., 2014a,b; Sham et al., 2015). In several clinical trials in patients with acute bacterial skin and skin structure infections (ABSSSI), 200 mg of TZD administered once daily for 6 days showed non-inferiority to 600 mg of LZD administered twice daily for 10 days (Prokocimer et al., 2013; Moran et al., 2014; Shor et al., 2015) and had fewer side effects. Based on the results, TZD was approved in the treatment of patients with ABSSSI in the United States and Europe. Despite the fact that TZD and LZD penetrated both, the ELF and the alveolar macrophages in the lungs, there are few studies regarding the efficacy of TZD in pulmonary infections, including pneumonia (Tessier et al., 2012; Lepak et al., 2012). The purpose of this study was to demonstrate the in-vivo efficacy of TZD in a murine model of hematogenous pulmonary infection caused by MRSA.

2. Material and methods

2.1. Bacterial strain

The MRSA strain used in this study was NUMR101, a clinical isolate obtained from the blood sample of a patient at the Nagasaki University Hospital (Yanagihara et al., 2008). The genetic characteristic of NUMR101 was identified by real-time polymerase chain reaction (PCR) using the same method as described in a previous report (Motoshima et al., 2010). The multilocus sequence typing (MLST) was performed according to the previous study (Enright et al., 2000). Sequence types (STs) were assigned to clusters using the MLST database (http://www.mlst.net). The bacteria were stored at −80 °C in a Microbank® bead preservation system (Pro-Lab Diagnostics, Ontario, CA) until use.

2.2. Antimicrobial agents

TZD was supplied by Bayer HealthCare AG, (Leverkusen, Germany). LZD injection 2 mg/ml and vancomycin (VAN) powder for solution for infusion were purchased from Pfizer Inc., (Tokyo, Japan) and Shionogi & Co., LTD., (Osaka, Japan), respectively. TZD was diluted in dimethyl sulfoxide (DMSO) and stored at −20 °C until use. For the treatment of the MRSA infection in the murine model, TZD, which was dissolved in DMSO and VAN powder for solution for infusion were diluted in normal saline, which is equivalent to the fluid volumeof LZD injection. In an antimicrobial susceptibility test, LZD powder for solution was supplied by Pfizer, Inc., (Groton, CT). LZD powder for solution and TZD were weighed and diluted in DMSO.

2.3. Antimicrobial susceptibility test

We tested the minimum inhibitory concentrations of VAN, LZD and TZD against the NUMR101 strain by a micro-dilution method, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2012). We weighed the antimicrobial agents and diluted in DMSO at 1.6 mg/ml and performed a two-fold serial dilution of the 1.6 mg/ml stock in DMSO to obtain a 50X working solution. From the 50× stock dilution, we added 2 μl volume to a 96-well assay plate containing 98 μl of cation-adjusted, Mueller Hinton II broth (Becton Dickson and Company, Sparks, MD), with the NUMR101 strain premixed at 5×10^5 CFU/ml. We incubated the plates overnight at 37 °C with 5% CO_2 and analyzed them after incubation.

2.4. Animals

We purchased specific-pathogen-free ddY male mice (6-week-old, 25–30 g body weight) from Japan SLC, Inc., Shizuoka, Japan. The mice were housed in a pathogen-free environment and received sterile food and water in the Biomedical Research Center at Nagasaki University.

2.5. Inoculum

The method of inoculation has been previously reported (Sawai et al., 1997). Briefly, we cultured the MRSA strain overnight in the Mueller-Hinton II agar at 37 °C with 5% CO_2 in 100% humidity. After incubation, we suspended the bacteria in normal saline, centrifuged them at 3000 rpm at 4 °C for 10 min and further, re-suspended them in normal saline followed by dilution to a bacterial count of 5×10^9 CFU/ml. We mixed 10 ml of this suspension with 10 ml of 4% molten Noble agar (Difco Laboratories, Detroit, MI) at 45 °C. We placed 1.0 ml of the agar-bacterium suspension into a 1.0 ml syringe and rapidly injected it into 49 ml of rapidly stirred, ice-cooled normal saline via a 26-gauge needle. This resulted in the solidification of the agar droplets into beads of approximately 250 μm in diameter. The final bacterial count was 5×10^7 CFU/ml.

2.6. Murine model of hematogenous pulmonary infection

The Ethics Review Committee for Animal Experimentation approved all the experimental protocols used in this study. The method used for inducing infection has been reported previously (Sawai et al., 1997). Briefly, we injected 0.25 ml of the suspension containing agar beads with a bacterial count of 1.25×10^7 CFU/mice, into the tail vein of the mice. After 24 h-post inoculation, a septic embolous of *Staphylococcus aureus* enmeshed in agar beads was detected in the pulmonary artery with inflammatory cell accumulation in its wall (Sawai et al., 1997).

2.7. Treatment protocol

We used the antimicrobial agents for the treatment, 24 h post-inoculation, at an interval of every 12 h (q12h) or every 24 h (q24h), by intra-peritoneal injection. We treated the mice with normal saline q12h (control), 25 mg/kg of VAN q12h (low-VAN), 110 mg/kg of VAN q12h (high-VAN), 120 mg/kg of LZD q12h or 20 mg/kg of TZD q24h. At the mentioned doses of these antimicrobial agents, the concentrations of these drugs after the ELF exposure in mice, were similar to those in humans, following intravenous regimens of 1 g of VAN q12h, 600 mg of LZD q12h and 200 mg of TZD q24h (Tessier et al., 2012). We injected the dose of high-VAN in mice to simulate the area under the curve in the concentrations versus time plot for the estimation of the free drug in plasma, which is the unbound fraction of drug, observed following an intravenous regimen of 1 g of VAN q12h in humans (Crandon et al., 2010).

2.8. Histopathological and bacteriological examinations

The method of histopathological and bacteriological examinations had been previously described (Kihara et al., 2009; Harada et al., 2013). We sacrificed the mice at specific time intervals by cervical dislocation and subsequently, dissected them to remove the lungs under aseptic conditions. We fixed the lungs in 10% formalin neutral buffer (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and stained them with hematoxylin-eosin. We suspended the lungs used for the bacteriological analysis, in 1 ml of normal