***Title***

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

***Author names and affiliations***

Norihito Kaku[a], Yoshitomo Morinaga[1], Kazuaki Takeda[a, b], Kosuke Kosai[a], Naoki Uno[a], Hiroo Hasegawa[a], Taiga, Miyazaki[b, c], Koichi Izumikawa[c], Hiroshi Mukae[b] and Katsunori Yanagihara[a]

[a]Department of Laboratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki, Japan

[b]Second Department of Internal Medicine, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki, Japan

[c]Department of Infectious Diseases, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki, Japan

***Corresponding author***

Norihito Kaku

Department of Laboratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.

Tel: +81-95-819-7574

Fax: +81-95-819-7257

E-mail: kaku-ngs@umin.ac.jp

***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 hours 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 hours 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.

## *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 (Grudmann, 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; Zargoulidis, 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 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; Sham DF, et al., 2015; Locke, et al.; 2014b).

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.

## ***Material and methods***

*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., 2010). 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.

*Antimicrobial agents:*

TZD was supplied by Bayer HealthCare AG, (Leverkusen, Germany). LZD injection 2mg/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.

*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 1.6mg/ml stock in DMSO to obtain a 50X working solution. From the 50X 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 $5x10^5$ CFU/ml. We incubated the plates overnight at 37°C with 5% $CO_2$ and analyzed them after incubation.

*Animals:*

We purchased specific-pathogen-free ddY male mice (6-week-old, 25 to 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.

*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 $5x10^9$ 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 $5x10^7$ CFU/ml.

*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 $1.25x10^7$ CFU/mice, into the tail vein of the mice. After 24 hours-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).

*Treatment protocol:*

We used the antimicrobial agents for the treatment, 24 hours post-inoculation, at an interval of every 12 hours (q12h) or every 24 hours (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).

*Histopathological and bacteriological examinations:*

The method of histopathological and bacteriological examinations have 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 saline and homogenized it with a homogenizer (AS One Co., Osaka, Japan). We collected the blood by a right ventricular puncture, using heparin-coated syringes. Subsequently, we cultured the lungs and the blood quantitatively by serial dilutions in the Mueller-Hinton II agar plates. After overnight incubation, we evaluated the number of visible colonies. The lowest level of detectable bacterial count was $1x\ 10^2$ CFU/ml.

*Cytokine ELISA:*

We collected the blood by a right ventricular puncture, using heparin-coated syringes. We separated the plasma by centrifugation and assayed the concentrations of tumor necrosis factor-alpha (TNF-alpha), interleukin-1beta (IL-1beta), interleukin-6 (IL-6) and macrophage inflammatory protein 2 (MIP-2) in the plasma, using the mouse cytokine enzyme-linked immunosorbent assay (ELISA) test kit (R&D Systems, Minneapolis, MN).

*Statistical analysis:*

We used a statistical software package (StatMate V; ATMS Co., Ltd., Tokyo, Japan) for all the statistical comparisons and calculated the survival rates using the Kaplan-Meier method. We performed the survival analysis using the log-rank test and expressed the data as mean and standard deviation (SD). In the graph of the bacterial count in the lungs and the blood, we depicted the data by the box-and-whisker plot and analyzed the differences between the groups using the one-way analysis of variance with the Tukey's post-hoc test. All the tests of significance were two-tailed and the alpha level for denoting statistical significance was set at $< 0.05$.

## *Results*

*Genetic characteristics of the NUMR101:*

The staphylococcal cassette chromosome *mec* (SCC*mec*) of the strain was type II. The strain carried virulence genes such as *sec* and *tsst* but did not carry *etb* and *pvl* genes. In the MLST, the allelic profile of *arcC, aroE, glpF, gmk, pta, tpi* and *yqiL* were 1, 4, 1, 4, 12, 1, 10, respectively. The sequence type of the NUMR101 strain was 5.

*MICs of the antimicrobial agents against the NUMR101:*

The MICs of VAN, LZD and TZD against the bacterial strain were 1.0, 0.5, and 1.0 mg/L, respectively.

*Murine model of hematogenous pulmonary infection:*

At the beginning of the treatment, 24 hours post-inoculation, the bacterial count in the lungs of the mice was 8.39 ± 0.35 $\log_{10}$ CFU/ml (n=6). Simultaneously, during the histopathological examination conducted by microscopy (n=3), many abscess lesions with the central bacterial colony zone (CBCZ) were observed, surrounded by inflammatory cells (Fig. 1).

*Therapeutic effects of antimicrobial agents on survival rate:*

In the survival study, the mice were treated by the prescribed methods until 120 hours post-inoculation and the survival rates were observed until 132 hours post-inoculation (n=6 in each group). The survival rates were significantly higher in the LZD- and the TZD-treatment groups at 83.3% and 100%, respectively, than those in the low-VAN treatment group (0.0%) ($P$ = 0.002 versus LZD and $P$ < 0.001 versus TZD) and the control group (16.7%) ($P$ = 0.010 versus LZD and $P$ = 0.004 versus TZD) (Fig. 2).

High-VAN could improve the survival rate by 50.0% but there were no significant differences when the high-VAN treatment group was compared with the control and the low-VAN treatment group.

*Histopathological and bacteriological examinations:*

The mice were sacrificed 36 hours post-inoculation i.e., 12 hours after the initial treatment. The lung specimens were used for the histopathological examination (n=4 in each group). In both the LZD- and the TZD-treatment groups, the area of the CBCZ had decreased, whereas, especially in the TZD treatment group the CBCZ had been eliminated from many abscess lesions (Fig. 3e). In contrast, such abscesses without the CBCZ were few in the low-VAN-, the high-VAN-, and the LZD-treatment groups (Fig. 3b, c, d). In the LZD treatment group, the area of the CBCZ had decreased, but had not been eliminated.

Simultaneously, the bacterial count in the lungs and the blood were evaluated (n=5 in each group). The bacterial count in the lungs had significantly decreased in the LZD- and the TZD-treatment groups to 7.47 ± 0.37 $\log_{10}$ CFU/ml ($P < 0.05$ versus. control) and 7.23 ± 0.81 $\log_{10}$ CFU/ml ($P < 0.05$ versus control), respectively, whereas, the control showed a count of 8.17 ± 0.16 $\log_{10}$ CFU/ml (Fig. 4a). In contrast, there were no statistically significant differences in the low-VAN- and the high-VAN treatment groups, showing a bacterial count of 8.03 ± 0.21 CFU/ml and 7.87 ± 0.24 $\log_{10}$ CFU/ml, respectively, when compared with the control group. The bacterial count in the blood had significantly decreased in the high-VAN- and the TZD-treatment groups showing a count of 2.88 ± 0.67 CFU/ml and 2.95 ± 0.75 $\log_{10}$ CFU/ml, respectively, in comparison with the control group (4.51 ± 0.42 $\log_{10}$ CFU/ml; $P < 0.05$ versus high-VAN- and TZD-treatment groups) (Fig. 4b). In comparison with the control group, the low-VAN and

LZD did not decrease the bacterial count in the blood, showing a count of 4.33 ± 1.18 $\log_{10}$ CFU/ml and 3.69 ± 0.49 $\log_{10}$ CFU/ml, respectively.

Since there was a conflict between the histopathological evaluation and the bacteriological examination in the lungs in the LZD treatment group at 36 hours post-inoculation, the mice were sacrificed and the lung specimens were used for histopathological examination at 60 hours post-inoculation (n=3 in each group). During this time, the CBCZ had vanished from many abscess lesions in the LZD- and the TZD-treatment groups (Fig. 5d, e).

*Inflammatory cytokines in the plasma:*

To eliminate the influence of the decrease in the number of bacteria, the mice were sacrificed 26 hours post-inoculation i.e., 2 hours after the initial treatment. The blood was collected by a right ventricular puncture, using heparin-coated syringes, and the plasma concentrations of TNF-alpha, IL-1beta, IL-6 and MIP-2 were evaluated by ELISA (n=5 in each group). Although there were no significant differences in the bacterial count in the lungs and the blood between all the groups during this time (data are not shown), the plasma concentrations of TNF-alpha, IL-6 and MIP-2 significantly decreased in the LZD- and the TZD-treatment groups in comparison with the control (Fig. 6a, c, d). There were no differences in the plasma concentration of IL-1beta (Fig. 6b). In the high-VAN treatment group, only the concentration of IL-6 significantly decreased in comparison with the control (Fig. 6c).

## *Discussion*

In this study, we used a murine model of hematogenous pulmonary infection to assess the effect of the antimicrobial agents on MRSA. Our previous study demonstrated the superiority of LZD over VAN against MRSA (NUMR101), vancomycin-insensitive *S. aureus* (VISA) or PVL-positive CA-MRSA (Yanagihara, et al., 2002; Yanagihara, et al., 2009). In this study, amongst all the antimicrobial agents, LZD and TZD significantly improved the survival rate, bacterial count in the lungs and the blood, and histopathological results, compared to the control. In contrast, low-VAN did not show an improvement for the same parameters. High-VAN significantly improved the bacterial count in the blood, but could not improve the other evaluation parameters.

In comparison to low-VAN, LZD and TZD significantly improved the survival rates and the bacterial count in the lungs. The studied doses of low-VAN, LZD and TZD showed similar pharmacokinetics to those after ELF exposures 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). Since the mice ELF exposures ($AUC_{0\text{-}24}$) for LZD was approximately 9-fold higher than that for low-VAN (Tessier, et al., 2012), LZD showed superior efficacy to low-VAN. On the other hand, the mice ELF exposures ($AUC_{0\text{-}24}$) for TZD was almost equal to that of low-VAN. Considering that the MICs of VAN and TZD were same, TZD might have a more potent antimicrobial activity than VAN.

The dose of high-VAN was selected to simulate the area under the curve in the concentration-time plot for estimation of the free drug in the plasma i.e., the unbound fraction of the drug, observed following an intravenous regimen of 1 g of VAN q12h in humans (Crandon, et al., 2010). Compared with the control and the low-VAN treatment

groups, high-VAN significantly reduced the bacterial count in the blood. However, high-VAN could not reduce the bacterial count in the lungs and the number of abscesses with the CBCZ observed during the histopathological analysis, compared with the control and the low-VAN treatment group. Based on these results, high-VAN was effective in the treatment of MRSA-induced bacteremia, but not in the treatment of lung abscesses. Furthermore, compared with LZD and TZD, high-VAN could not improve the survival rate, the bacterial count in the lungs and the number of abscesses with the CBCZ observed during the histopathological analysis. Thus, LZD and TZD were more effective than high-VAN in the murine model of hematogenous pulmonary infection.

There were almost no significant differences in the antimicrobial effect between the LZD- and the TZD-treatment groups. There were no differences between the LZD- and the TZD-treatment groups in the reduction of the bacterial count in the lungs and the blood. Additionally, at 36 hours after initial treatment, LZD as well as TZD vanished the CBCZ from many abscess lesions. However, during the histopathological analysis at 12 hours after the initial treatment, slight differences were observed between the both drugs. TZD could eliminate the CBCZ from many lung abscesses, whereas LZD could not.

In addition to the comparison of the antimicrobial effects of the drugs, the plasma concentrations of the inflammatory cytokines were also compared in this study. At 2 hours after initial treatment, LZD and TZD significantly reduced the plasma concentrations of TNF-alpha, IL-6, and MIP-2 in comparison with control. Previous studies have reported that LZD reduced the lipopolysaccharide-induced cytokine production from peripheral blood mononuclear cells directly (Garcia-Roca, et al., 2006; Lambers, et al., 2010; Takahashi, et al, 2010). Such a direct immunomodulatory effect of LZD was observed in the airway epithelial cells, as well (Kaku, et al., 2014). Since there were no significant differences in the bacterial count in the lungs and the blood

between the treatment groups at 2 hours after initial treatment, we assume that LZD and TZD showed direct immunomodulatory effect in this study. However, some studies reported that the inhibition of the MRSA-induced inflammatory cytokines production by LZD was associated with the reduction in the bacterial count or toxin production (Yoshizawa, et al., 2012; Sharma-Kuinkel, et al., 2013; Zargoulidis, et al., 2012; Yanagihara K, et al., 2002). Therefore, there is a possibility that LZD and TZD showed indirect immunomodulatory effect associated with the reduction in the toxin production.

There were some limitations in this study. First, the mouse species were different from the previous study on pharmacokinetic analysis of low-VAN, LZD, and TZD (Tessier, et al., 2012). Second, both mouse species and route of administrations were different from the previous study on pharmacokinetic analysis of high-VAN (Crandon, et al., 2010). Since there is a possibility that these differences differ the pharmacokinetic results of antimicrobial agents in this study from the previous studies, pharmacokinetic analysis in this model are needed. Third, we used only one clinical strain in this study. Fourth, it remains unknown whether LZD and TZD cured the mouse or not because we examined until a short period of time. Finally, the mechanisms of the immunomodulatory effect of LZD and TZD remain unknown. Accordingly, more experimental studies are needed to confirm the effects of TZD in a pulmonary infection.

***Conclusions***

In conclusion, we showed that TZD, a second-generation oxazolidinone, had antimicrobial and immunomodulatory effects in a murine model of hematogenous pulmonary infection caused by MRSA. Thus, TZD could be considered to be clinically effective in patients with pulmonary infection caused by MRSA.

339

340

**Acknowledgements**

*Funding:* This work was supported by Bayer HealthCare AG, (Leverkusen, Germany) and a Grant-in-Aid for Young Scientists (B) (No. 25860830 to Y.M.) from the Japan Society for the Promotion of Science.

*Competing interests*: None declared.

*Ethical approval*: This study was approved by the Ethics Review Committee of Nagasaki University for Animal Experimentation.

## References


Crandon JL, Kuti JL, Nicolau DP. Comparative efficacies of human simulated exposures of telavancin and vancomycin against methicillin-resistant *Staphylococcus aureus* with a range of vancomycin MICs in a murine pneumonia model. 2010. Antimicrob Agents Chemother. 54, 5115-9.

Clinical and Laboratory Standards Institute, 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standards, ninth edition, M07-A9CLSI, Wayne, PA.

Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. 2000. J Clin Microbiol. 38, 1008-15.

Garcia-Roca P, Mancilla-Ramirez J, Santos-Segura A, Fernández-Avilés M, Calderon-Jaimes E. 2006. Linezolid diminishes inflammatory cytokine production from human peripheral blood mononuclear cells. Arch Med Res. 37, 31-5.

Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. 2006. Emergence and resurgence of methicillin-resistant *Staphylococcus aureus* as a public-health threat. Lancet. 368, 874-85.

Gu B, Kelesidis T, Tsiodras S, Hindler J, Humphries RM. 2013. The emerging problem of linezolid-resistant *Staphylococcus*. J Antimicrob Chemother. 68, 4-11.

Harada Y, Yanagihara K, Yamada K, Migiyama Y, Nagaoka K, Morinaga Y, et al. 2013. *In vivo* efficacy of daptomycin against methicillin-resistant *Staphylococcus aureus* in a murine model of hematogenous pulmonary infection. Antimicrob Agents Chemother. 57, 2841-4.

Holzknecht BJ, Hardottir H, Haraldsson G, Westh H, Valsdottir F, Boyce K, et al. 2010. Changing epidemiology of methicillin-resistant *Staphylococcus aureus* in Iceland from

374 2000 to 2008: a challenge to current guidelines. J Clin Microbiol. 48, 4221-7.
375 Kaku N, Yanagihara K, Morinaga Y, Yamada K, Harada Y, Migiyama Y, et al. 2014.
376 Immunomodulatory effect of linezolid on methicillin-resistant *Staphylococcus aureus*
377 supernatant-induced MUC5AC overexpression in human airway epithelial cells.
378 Antimicrob Agents Chemother. 58, 4131-7.
379 Kihara R, Yanagihara K, Morinaga Y, Araki N, Nakamura S, Seki M, et al. 2009.
380 Potency of SMP-601, a novel carbapenem, in hematogenous murine bronchopneumonia
381 caused by methicillin-resistant and vancomycin-intermediate *Staphylococcus aureus*.
382 Antimicrob Agents Chemother. 52, 2164-8.
383 Lambers C, Burian B, Binder P, Ankersmit HJ, Wagner C, Müller M, et al. 2010. Early
384 immunomodulatory effects of linezolid in a human whole blood endotoxin model. Int J
385 Clin Pharmacol. 48, 419-24.
386 Lepak AJ, Marchillo K, Pichereau S, Craig WA, Andes DR. Comparative
387 pharmacodynamics of the new oxazolidinone tedizolid phosphate and linezolid in a
388 neutropenic murine *Staphylococcus aureus* pneumonia model. 2012. Antimicrob Agents
389 Chemother. 56, 5916-22.
390 Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, et al. 2012.
391 Infectious Diseases Society of America. Clinical Practice guidelines by the Infectious
392 Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus*
393 *aureus* infections in adults and children. Clin Infect Dis. 52, e18-55.
394 Locke JB, Zurenko GE, Shaw KJ, Bartizal K. 2014a. Tedizolid for management of
395 human infections: *in vitro* characteristics. Clin infect Dis. 58 Suppl 1, S35-42.
396 Locke JB, Zuill DE, Scharn CR, Deane J, Sham DF, Goering RV, et al.
397 2014b.Identification and characterization of linezolid-resistant *cfr*-positive
398 *Staphylococcus aureus* USA300 isolates from a New York City medical center.

Antimicrob Agents Chemother. 58, 6949-52.

Moran GJ, Fnag E, Corey GR, Das AF, De Anda C, Prokocimer P.2014. Tedizolid for 6 days versus linezolid for 10 days for acute bacterial skin and skin-structure infections (ESTABLISH-2): a randomized, double-blind phase3, non-inferiority trial. Lancet Infect Dis. 14, 696-705.

Motoshima M, Yanagihara K, Morinaga Y, Matsuda J, Sugahara K, Yamada Y, et al. 2010. Genetic diagnosis of community-acquired MRSA: a multiplex real-time PCR method for staphylococcal cassette chromosome mec typing and detecting toxin genes. Tohoku J Exp Med. 220, 165-70.

Popovich KJ, Weinstein RA, Hota B. 2008. Are community-associated *Staphylococcus aureus* (MRSA) strains replacing traditional nosocomial MRSA strains? Clin Infect Dis. 46, 787-94.

Prokocimer P, De Anda C, Fang E, Mehra P, Das A. 2013. Tedizolid phosphate vs linezolid for treatment of acute bacterial skin and skin structure infections: the ESTABLISH-1 randomized trial. JAMA. 309, 559-69.

Rodvold KA, McConeghy KW. 2014. Methicillin-resistant *Staphylococcus aureus* therapy: past, present, and future. Clin Infect Dis. 58 Suppl 1, S20-7.

Sawai T, Tomono K, Yanagihara K, Yamamoto Y, Kaku M, Hirakata Y, et al. 1997. Role of coagulase in a murine model of hematogenous pulmonary infection induced by intravenous injection of *Staphylococcus aureus* enmeshed in agar beads. Infect Immun. 65, 466-71.

Sham DF, Deane J, Bien PA, Locke JB, Zuill DE, Shaw KJ, Bartizal KF. 2015. Results of the surveillance of tedizolid activity and resistance program: *in vitro* susceptibility of gram-positive pathogens collected in 2011 and 2012 from the United States and Europe. Diagn Microbiol Infect Dis. 81, 112-8.

424 Sharma-Kuinkel BK, Zhang Y, Yan Q, Ahn SH, Fowler VG Jr. 2013. Host gene
425 expression profiling and *in vivo* cytokine studies to characterize the role of linezolid and
426 vancomycin in methicillin-resistant Staphylococcus aureus (MRSA) murine sepsis
427 model. PLoS One. 8, e60463.
428 Shor AF, Lodise TP, Corey GR, De Anda C, Fang E, Das AF, et al. 2015. Analysis of the
429 phase 3 ESTABLISH trials of tedizolid versus linezolid in acute bacterial skin and skin
430 structure infections. Antimicrob Agents Chemother. 59, 864-71.
431 Takahashi G, Sato N, Yaegashi Y, Kojika M, Matsumoto N, Kikkawa T, et al. 2010.
432 Effect of linezolid on cytokine production capacity and plasma endotoxin levels in
433 response to lipopolysaccharide stimulation of whole blood. J Infect Chemother. 16,
434 94-9.
435 Tessier PR, Keel RA, Hagihara M, Crandon JL, Nicolau DP. 2012. Comparative *in vivo*
436 efficacies of epithelial lining fluid exposures of tedizolid, linezolid, and vancomycin for
437 methicillin-resistant *Staphylococcus aureus* in a mouse pneumonia model. Antimicrob
438 Agents Chemother. 56, 2342-6.
439 Yanagihara K, Kaneko Y, Sawai T, Miyazaki Y, Tsukamoto K, Hirakata Y, et al. 2002.
440 Efficacy of linezolid against methicillin-resistant or vancomycin-insensitive
441 *Staphylococcus aureus* in a model of hematogenous pulmonary infection. Antimicrob
442 Agents Chemother. 46; 3288-91.
443 Yanagihara K, Morinaga Y, Nakamura S, Seki M, Izumikawa K, Kakeya H, et al. 2008.
444 Subinhibitory concentrations of telithromycin, clarithromycin and azithromycin reduce
445 methicillin-resistant *Staphylococcus aureus* coagulase *in vitro* and in *vivo*. J Antimicrob
446 Chemother. 61, 647-50.
447 Yanagihara K, Kihara R, Araki N, Morinaga Y, Seki M, Izumikawa K, et al. 2009.
448 Efficacy of linezolid against panton-valentine leukocidin (PVL)-positive

449 methicillin-resistant *Staphylococcus aureus* (MRSA) in a mouse model of
450 hematogenous pulmonary infection. Int J Antimicrob Agnets. 34, 477-81.
451 Yoshizawa S, Tateda K, Saga T, Ishii Y, Yamaguchi K. 2012. Virulence-suppressing
452 effects of linezolid on methicillin-resistant *Staphylococcus aureus*: possible contribution
453 to early defervescence. Antimicrob Agents Chemothe. 56, 1744-1748.
454 Zarogoulidis P, Papanas N, Kloumis I, Chatzaki E, Maltezos E, Zarogoulidis K. 2012.
455 Macrolides: from in vitro anti-inflammatory and immunomodulatory properties to
456 clinical practice in respiratory diseases. Eur J Clin Pharmacol. 68, 479-503.

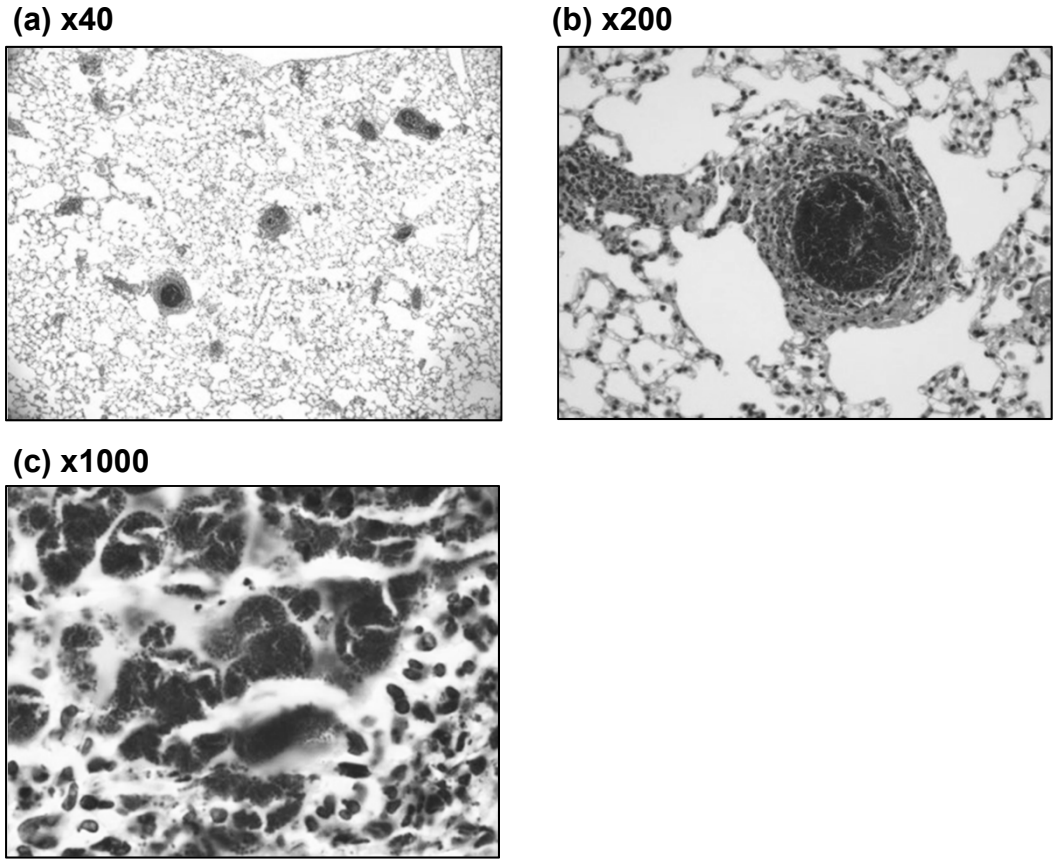


**Fig. 1. Histopathological examination of the lungs before the start of the treatment:** Representative data from each group, at 24 hours post-inoculation of NUMR101 are shown (*n* = 3). Many abscess lesions with the central bacterial colony zones (CBCZ) surrounded by the inflammatory cells were observed. Hematoxylin and eosin stain; original magnification, x40 (a), x200 (b) and x1000 (c)

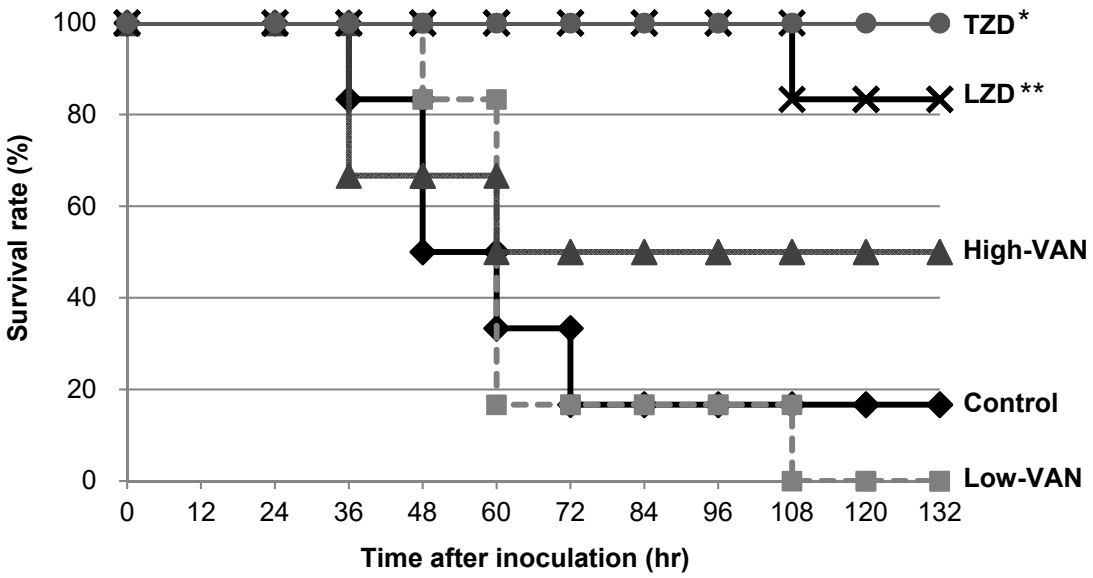


**Fig. 2. Effects of the antimicrobial agent on the survival rate:**
The mice were treated with normal saline q12h (control), 25 mg/kg of vancomycin q12h (low-VAN), 110 mg/kg of vancomycin q12h (high-VAN), 120mg/kg of linezolid q12h (LZD) or 20 mg/kg of tedizolid q24h (TZD). The survival rates were observed until 132 hours post-inoculation ($n$ = 6 in each group). The survival rates in the TZD- and LZD-treatment groups were significantly higher than those in the control and the low-VAN groups. Asterisk, $P$ = 0.004 versus control and $P$ < 0.001 versus low-VAN for the TZD treatment group. Double asterisk, $P$ = 0.010 versus control and $P$ = 0.002 versus low-VAN for the LZD treatment group.

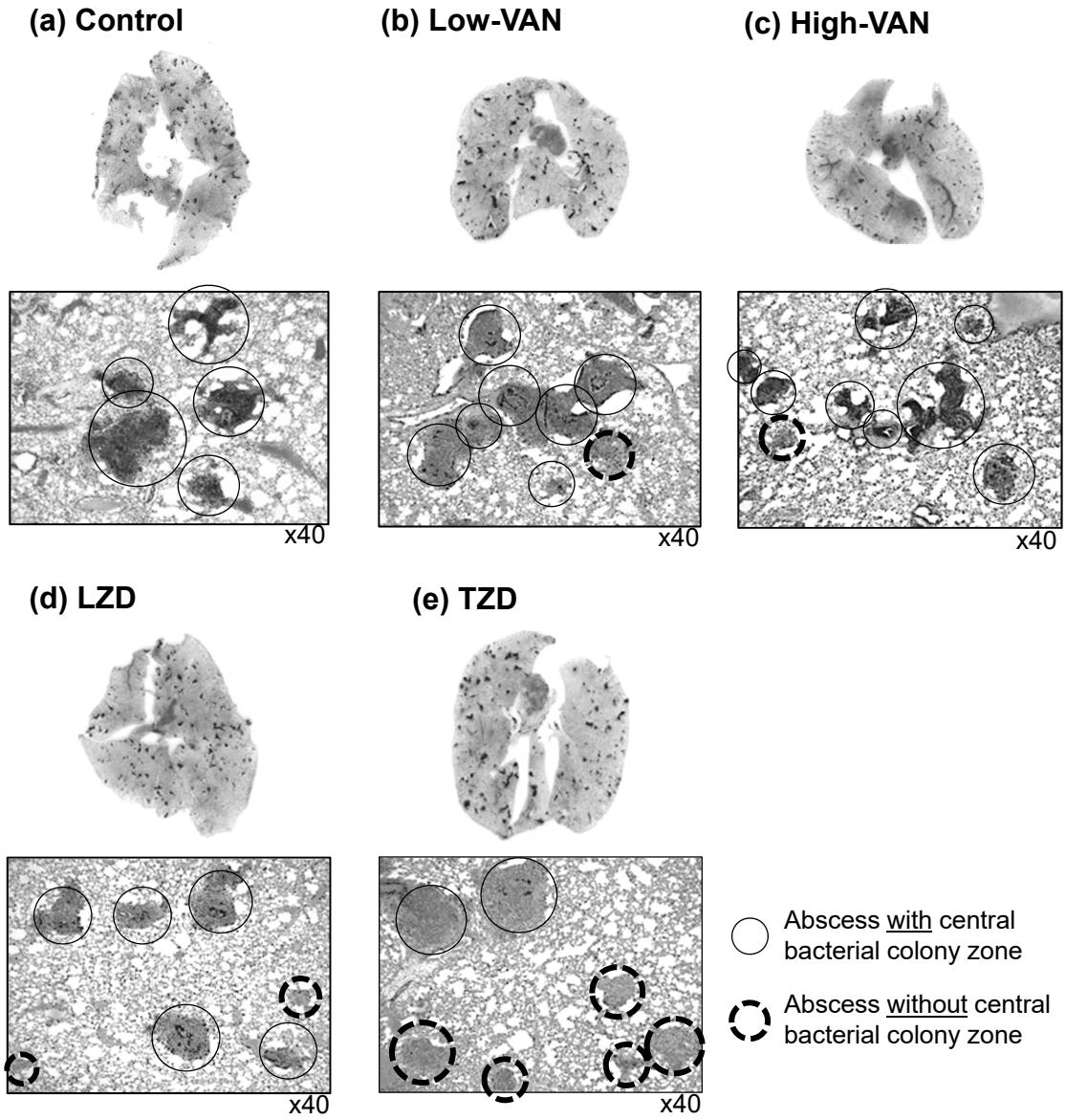


**Fig. 3. Histopathological examination of the lung specimens 12 hours after the initial treatment:**
The mice were treated once with normal saline (control), 25 mg/kg of vancomycin (low-VAN), 110 mg/kg of vancomycin (high-VAN), 120 mg/kg of linezolid (LZD) or 20 mg/kg of tedizolid (TZD). Twelve hours after the initial treatment, the mice were sacrificed. The representative data are shown ($n$ = 4). In the LZD- and the TZD-treatment groups, the area of the central bacterial colony zone (CBCZ) decreased (d), (e). Especially in the TZD treatment group, the CBCZ was eliminated from many abscess lesions (e). In contrast, such abscesses without CBCZ were few in the low-VAN treatment group (b) and the high-VAN treatment group (c).

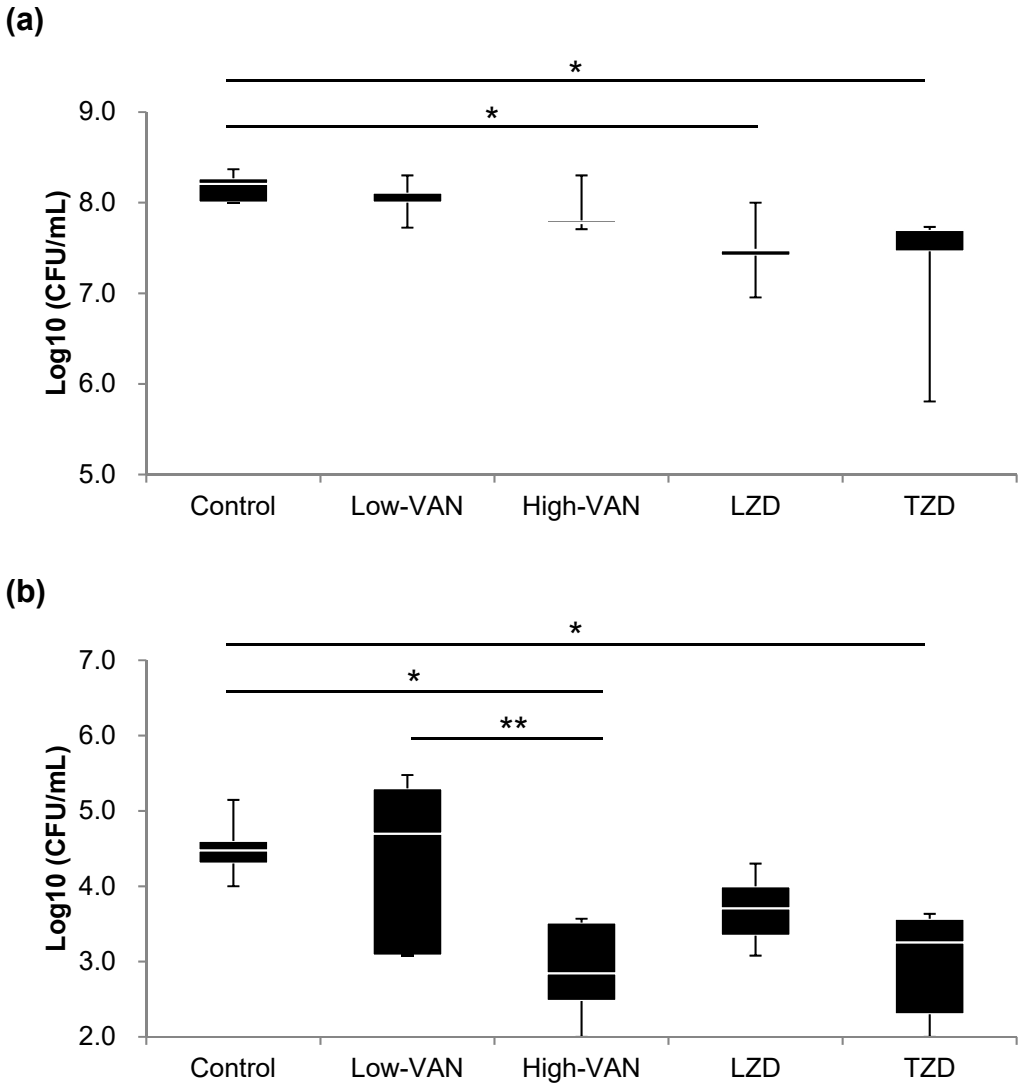


**Fig. 4. Bacterial count in the lungs and the blood:**
The mice were treated with normal saline q12h (control), 25 mg/kg of vancomycin q12h (low-VAN), 110 mg/kg of vancomycin q12h (high-VAN), 120 mg/kg of linezolid q12h (LZD), or 20 mg/kg of tedizolid q24h (TZD). Twelve hours after the initial treatment, the mice were sacrificed and the bacterial count in the lungs (a) and the blood (b) were analyzed ($n$ =5). Box-and-whisker plots show the range and median of the bacterial count. LZD and TZD significantly decreased the bacterial count in the lungs compared with. The bacterial count in the blood was significantly decreased by high-VAN and TZD in comparison with the control. Additionally, only high-VAN significantly decreased the bacterial count in the blood in comparison with low-VAN. Asterisk, $P < 0.05$ versus control. Double asterisk, $P < 0.05$ versus low-VAN

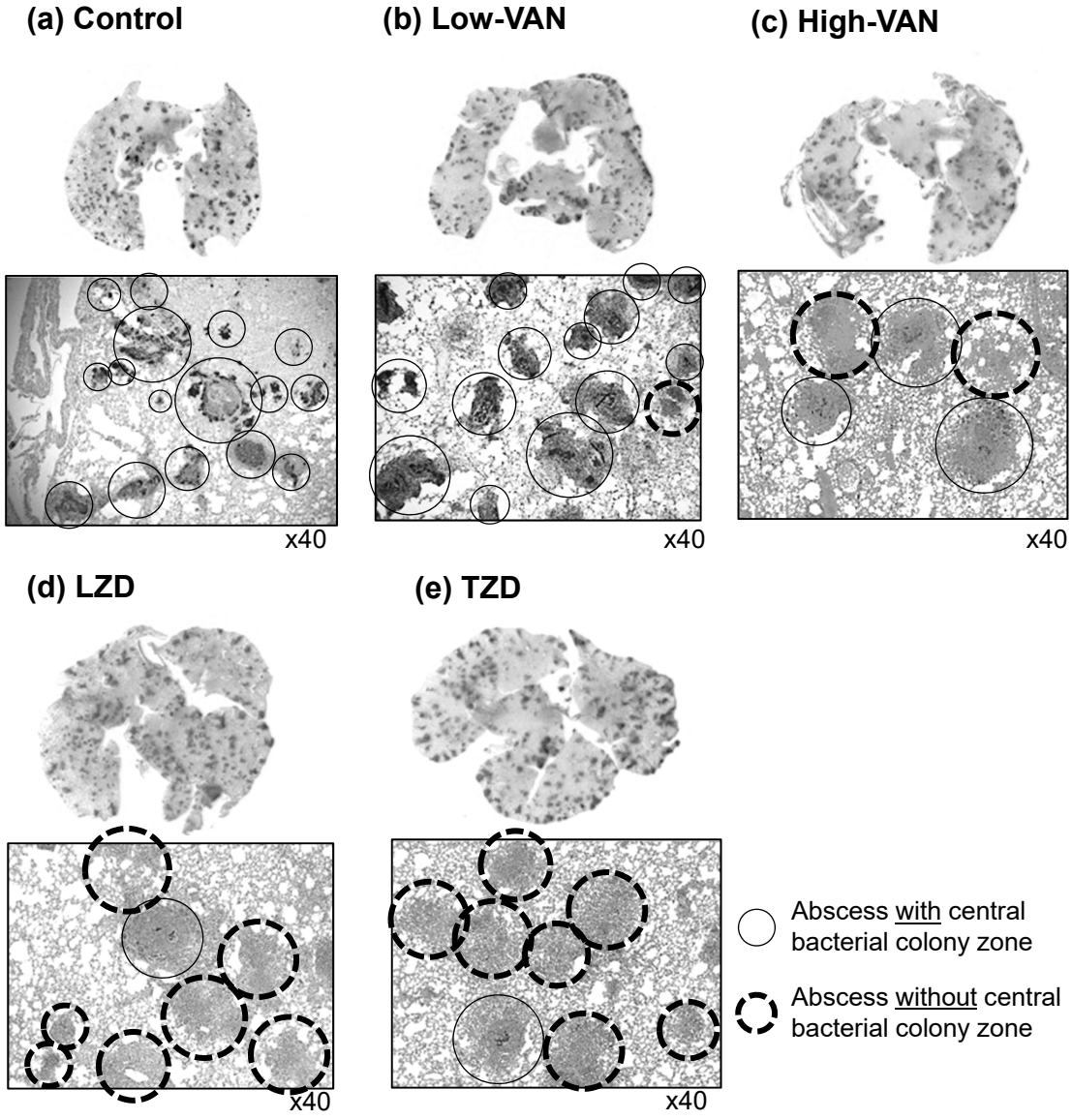


**Fig. 5. Histopathological examination of the lung specimens at 60 hours post-inoculation:**
The mice were treated with normal saline q12h (control), 25 mg/kg of vancomycin q12h (low-VAN), 110 mg/kg of vancomycin q12h (high-VAN), 120 mg/kg of linezolid q12h (LZD) or 20 mg/kg of tedizolid q24h (TZD). Sixty hours post-inoculation, the mice were sacrificed. The representative data are shown ($n$ = 3). In the LZD- as well as the TZD-treatment groups, the central bacterial colony zones (CBCZ) were eliminated from many abscess lesions (d, e). In contrast, such abscesses without the CBCZ were few in the low-VAN- (b) and the high-VAN-treatment groups (c).

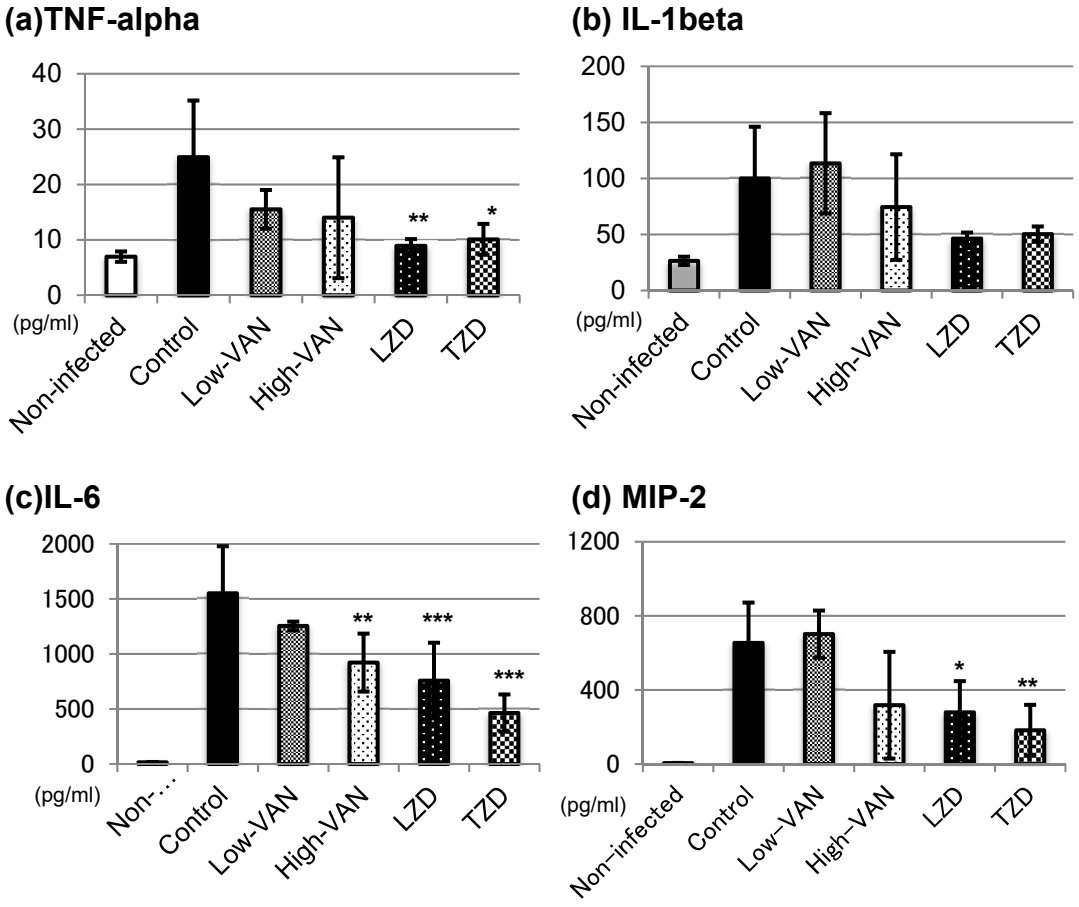


**Fig. 6. Plasma concentrations of the inflammatory cytokines:**
The mice were treated with normal saline q12h (control), 25 mg/kg of vancomycin q12h (low-VAN), 110 mg/kg of vancomycin q12h (high-VAN), 120 mg/kg of linezolid q12h (LZD) or 20 mg/kg of tedizolid q24h (TZD). The plasma concentrations of the inflammatory cytokines were evaluated at 26 hours post-inoculation i.e., 2 hours after the initial treatment (n=5 in each group). LZD and TZD significantly improved the plasma concentrations of TNF-alpha, IL-6 and MIP-2 in comparison with the (a, c, d). There were no significant differences in IL-1beta (b). High-VAN significantly improved the concentrations of IL-6 (c). Asterisk, $P < 0.05$, versus control. Double asterisk, $P < 0.01$, versus control. Triple asterisk, $P < 0.001$ versus control.