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Note

Comparative efficacies of daptomycin, vancomycin, and linezolid in experimental enterococcal peritonitis

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ABSTRACT

Enterococci have become increasingly important pathogens for nosocomial infection (e.g. bacteremia, intra-abdominal infection, endocarditis, etc.), related to their intrinsic resistance to many antibiotics. Although the *in vitro* susceptibility of daptomycin (DAP) against *Enterococci* is well established, the Food and Drug Administration has only approved its use for complicated skin and skin structure infections induced by *Enterococcus faecalis*. In this study we evaluated the potential therapeutic application of DAP in a murine model of enterococcal experimental peritonitis. Mice were injected intraperitoneally with 4×10^{10} colony-forming units of *Enterococcus faecium*. DAP alone, DAP combined with ampicillin, vancomycin, or linezolid were administered 2 h after enterococcal inoculation and examined the survival, viable bacteria counts, the level of KC/CXCL1 in the peritoneal fluid. The viable bacteria counts in the peritoneal fluid of the DAP− or DAP plus ampicillin-treated groups were decreased significantly compared to those of the vancomycin- and linezolid-treated groups ($P < 0.05$) at 6 and 12 h after the inoculation of *Enterococcus*. The level of neutrophil chemoattractants KC in the peritoneal fluid at 12 h after enterococcal inoculation was significantly decreased in the DAP plus ampicillin-treated group ($P < 0.05$). In addition DAP showed the inhibitory effect of enterococcal biofilm formation dose-dependently by a microtiter biofilm assay. These results indicate that DAP, particularly with β-lactams, is a possible alternative agent to treat severe enterococcal infection such as peritonitis.

Enterococcus faecalis and *Enterococcus faecium* have become particularly important etiological pathogens for nosocomial infections, as they can survive in hospital environments and colonize patients, causing infections such as urinary tract infections, hepatobiliary sepsis, endocarditis, surgical wound infections, bacteremia, and neonatal sepsis [1]. Enterococci have recently become one of the most common nosocomial pathogens, mainly owing to the increased use of antineoplastic, biological, and other immunosuppressive agents [2,3]. Enterococcal intra-abdominal infection is particularly common in nosocomial situations, especially post-surgery. Previous studies revealed that enterococci are the common causative pathogens for intra-abdominal infections after pancreaticoduodenectomy and in liver transplant recipients [4]. Vancomycin (VAN) has been mainly used for the empirical treatment of enterococcal infections since *E. faecium* emerged as a multi-resistant pathogen. Daptomycin (DAP) is a lipopeptide antibiotic with bactericidal activity against a broad range of Gram-positive bacteria, including *Enterococcus* spp., both *in vitro* and *in vivo*. DAP

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is indicated by the Food and Drug Administration [5] for the treatment of complicated skin and skin structure infections, as well as for bloodstream infections by *Staphylococcus aureus*, including that in patients with right-sided infective endocarditis. In the presence of peritoneal fluid, the mean concentration of VAN was found to be only 15% of the serum concentration [6], indicating that it does not effectively penetrate into the peritoneal fluid. Previous studies have demonstrated the clinical efficacies of DAP against enterococcal peritonitis [7]; however, few studies have examined the effectiveness of DAP in enterococcal intra-abdominal infections. Therefore, the aim of this study was to examine the efficacy of DAP compared with that of the existing anti-Gram positive agents VAN and linezolid (LZD) for treating enterococcal peritonitis in a murine model.

Specific pathogen-free, 10-week-old, female wild-type C57BL/6 mice were purchased from SLC, Inc. (Shizuoka, Japan). The Ethics Review Committee for Animal Experimentation approved all experimental protocols used in this study. All mice were bred in the animal facility of the Academic Medical Center (Nagasaki, Japan). A VAN-, LZD-, and DAP-susceptible *E. faecium* (strain 2010-6186) clinically isolated from Nagasaki University Hospital was used for the *in vivo* study. The MICs of daptomycin, vancomycin and linezolid against *E. faecium* (strain 2010-6186) were 1, 1 and 2 μg/ml, respectively. Mice were injected intraperitoneally with 4×10^{10} colony-forming units (CFU) of *E. faecium* (strain 2010-6186) in 200 μL of sterile isotonic saline [8] and *treated with* DAP [50 mg/kg per mouse, q24h, intravenous (i.v.)], and with DAP combined with ampicillin [AMP, 100 mg/kg per mouse, q24h, intraperitoneal (i.p.)], VAN, (25 mg/kg per mouse, q12h, i.v.), or LZD (25 mg/kg per mouse, q12h, i.v.) 2 h after enterococcal inoculation for 48 h [9–12]. The mice were euthanized 6, 12, and 48 h after infection, and the peritoneal fluids, blood, and spleen were harvested to count the viable bacteria [8]. Each sample was serially diluted by 10-fold using sterile saline, and 30 μL was plated onto brain heart infusion (BHI) agar plates (BD Biosciences, Franklin Lakes, NJ, USA) for quantitative culture. The plates were incubated at 37 °C under 5% CO_2, and CFU were counted after 24 h. The level of murine cytokine-induced neutrophil chemoattractant (KC) in peritoneal lavage fluid was measured 12 h after infection, by an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA). To examine the anti-biofilm activity of each agent, enterococcal biofilms were measured by a modified microtiter biofilm assay [13]. Bacteria cells were briefly suspended in BHI with Ca^{2+} (supplemented to a physiologic level of 50 mg/L Ca^{2+}) at an optical density (OD) of approximately 0.15 at 492 nm, and a 200-μL aliquot was inoculated into a well of a 96-well microplate coated with hydroxyapatite (MBEC™ Biofilm Inoculator, Innovotech, Inc., Edmonton, AB, Canada). Biofilm formation was confirmed after 48 h of culture, the medium was changed, and then 200 μL fresh BHI containing Ca^{2+} and antibiotics (DAP, VAN, or LZD) was added to each well with a dose-dependent minimum inhibitory concentration (MIC; 1000 × MIC, 10 × MIC, 0.1 × MIC). After 24 h of exposure to the antibiotics, biofilms were stained with 1% freshly adjusted crystal violet at room temperature for 15 min and washed three times with sterile water. After extraction with 230 μL of 99% ethanol, the absorbance of the biofilms was measured at OD_{540} using a microplate reader. Survival curves were compared by log-rank test. The differences in the means and standard deviations of bacterial counts and the levels of cytokines between groups were analyzed by one-way analysis of variance (ANOVA), followed by the Tukey post-hoc test. For the biofilm assay, four samples were tested in all experiments, and the average and standard deviation of each experiment were calculated and statistically compared to baseline values by one-way ANOVA. A value of $P < 0.05$ was considered significant. Statistical analyses were conducted using SPSS® v20 (SPSS Inc., Chicago, IL, USA).

Survival of the mice was observed for 48 h (Fig. 1; $n = 10$ per group). Treatment with DAP, DAP plus AMP, VAN, or LZD significantly prolonged the mean time until death compared to that in untreated animals; however, no significant differences were observed between each treatment group. As shown in Fig. 2a, The viable bacteria counts of the peritoneal fluid 6 h after inoculation in the DAP- or DAP plus AMP-treated group were significantly decreased compared with those in the VAN-treated groups ($P < 0.05$). Viable bacteria counts in the spleen of the DAP plus AMP-treated animals were significantly decreased compared to those in the LZD-treated group but not to those in the VAN-treated group. No significant difference was observed in the bloodstream. At 12 h, the viable bacteria counts of the peritoneal fluid in the DAP-, DAP plus AMP-, or LZD-treated groups were significantly decreased compared with those in the non-treated groups ($P < 0.05$). Viable bacteria counts in the spleen were only significantly decreased in the DAP plus AMP-treated animals compared to those in the non-treated groups. At 48 h after inoculation, there was no significant difference in the viable bacterial counts in each group. At 12 h after inoculation, the KC levels of the peritoneal fluid in the DAP plus AMP-treated group were significantly decreased compared to those in the non-treated groups (Fig. 2b). The biofilm inhibition effect was defined as the ratio of antibiotic-treated biofilm to untreated biofilm. As shown in Fig. 3, only DAP showed a significant biofilm inhibition effect compared to VAN at the 1000 × MIC administration ($P < 0.05$).

In this study, we used a murine model of enterococcal peritonitis and found that DAP, particularly in combination with AMP, was more effective in the early stages of treatment (6 and 12 h after commencing treatment) than LZD or VAN. The bactericidal action of DAP has been shown to produce improvements in the early stages of methicillin-resistant *S. aureus* (MRSA)-induced peritonitis, by fluoroluminescence in an animal experiment [14]. In addition, Sakoulas et al. [15] reported that the addition of AMP shifted the bacterial outer membrane towards a negative charge, reinforcing the bond with the positively charged DAP and accelerating its antimicrobial action. In this study, the survival rate and number of viable bacteria in the internal organs were compared 48 h after DAP and VAN and LZD treatment. Although no significant difference was observed, a decrease in the number of viable bacteria and KC in the peritoneal fluid was observed 6 and 12 h after inoculation in the DAP plus AMP-treated group. Therefore, the role of DAP may be significant for severe infections such as bacterial peritonitis, where early intervention and recognition of the clinical effects can influence the vital prognosis.

Despite reports showing that high doses of DAP are effective against severe infections, recent studies have shown that an

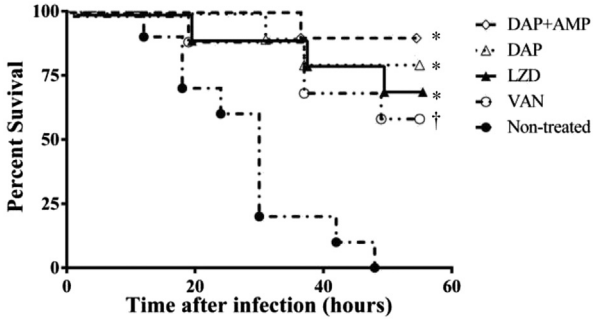

Fig. 1. Survival rate (percentage) of mice intraperitoneally infected with *E. faecium*. Mice were injected intraperitoneally with 4×10^{10} CFU of *E. faecium* and treated with daptomycin (DAP; 50 mg/kg per mouse, q24h, i.v.), DAP plus ampicillin (AMP) (100 mg/kg per mouse, q24h, i.p.), vancomycin (VAN; 25 mg/kg per mouse, q12h, i.v.), or linezolid (LZD; 25 mg/kg per mouse, q12h, i.v.). n = 10 mice per group. $^{*}P < 0.001$, $^{\dagger}P = 0.001$.