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Daptomycin versus Vancomycin in Treatment of Methicillin-Resistant *Staphylococcus aureus* Meningitis in an Experimental Rabbit Model

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In this study, we aimed to compare the antibacterial activities of daptomycin and vancomycin in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) meningitis (induced by MRSA strain ATCC 43300) in an experimental rabbit meningitis model. After an 8-h period of treatment, bacterial counts decreased significantly in both treatment groups compared to the control group ($P < 0.05$). However, there was no statistically significant difference between treatment groups. Our results suggest that the antibacterial activity of daptomycin is similar to vancomycin for treatment in the experimental MRSA meningitis model in rabbits.

Staphylococcus aureus can cause community-acquired and nosocomial bacterial meningitis and is associated with significant mortality. Methicillin-resistant *S. aureus* (MRSA) is one of the major etiologic agents in hospital-acquired central nervous system infections. Since the available treatment options are limited, therapy of MRSA meningitis is problematic (1). The aim of this study was to compare the efficacies of vancomycin and daptomycin in MRSA meningitis in an experimental rabbit meningitis model.

Bacterial strain. *S. aureus* ATCC 43300 (vancomycin MIC, 1 mg/liter; daptomycin MIC, 0.064 mg/liter [measured in duplicate using the Etest; AB Biodisk, Solna, Sweden]) was used as the infecting bacterial strain (2).

The bacterial solution was prepared in 0.9% NaCl at a concentration of 10^6 CFU/ml as described elsewhere (2, 3).

Antimicrobial agents. The drugs used were vancomycin (Lilly, Indianapolis, IN) and daptomycin (Novartis Pharma AG, Basel, Switzerland).

Rabbit meningitis model. New Zealand White rabbits weighing 2.5 to 3.0 kg were anesthetized by intramuscular injections of ketamine (35 mg/kg of body weight) and xylazine (5 mg/kg) before each intraventricular intervention, including induction of meningitis and cerebrospinal fluid (CSF) sampling (2). The duration of anesthesia was 10 to 15 min. Then, 0.5 ml of the bacterial solution of MRSA was injected directly into the cisterna magna of each rabbit by using a 22-gauge spinal needle (Hayat Ticaret, Istanbul, Turkey).

Animals were not anesthetized after the primary inoculation or between the CSF sampling procedures. In addition, they were kept in their cages, except during intraventricular interventions.

Sixteen hours after the inoculation, meningitis criteria were determined. CSF white cell counts of more than 1,000/mm^3 (counted by using a Thoma slide) and a bacterial count of $\geq 10^3$ CFU/ml were accepted as the indications of meningitis. Then, rabbits were separated into three groups: the daptomycin (D) group received one dose of 15 mg/kg daptomycin, and the vancomycin (V) group received 20 mg/kg vancomycin two times (4 h apart) through a peripheral ear vein. The control (C) group did not receive any drug treatment (4).

Quantitative bacterial cultures were prepared from CSF samples, which were obtained at the beginning and at 8 hours after treatment. CSF and serum drug levels were measured by a bioassay technique in samples obtained at 8 hours after drug treatment (2, 4). At the end of the study period (8 h after the initiation of drug treatment), animals were humanely killed by intravenous infusion of a high dose of nembutal.

Measurement of bacterial concentrations. Bacterial concentrations in the CSF were measured at 16 h after infection (at the end of the incubation period and before the first dose of vancomycin or daptomycin was administered) and 8 h after initiation of drug treatment by plating undiluted and serial 10-fold and 100-fold dilutions of CSF (20-μl volume) (5) on 5% sheep blood agar and incubated at 37°C for 24 h. The bacterial response was evaluated for three categories: full response, sterilization of CSF; partial response, any decrease in bacterial count; bacteriological failure, an increased bacterial count (2).

Antibiotic assay. Levels of daptomycin and vancomycin were measured twice by using a bioassay technique that involved *Kuceria rhizophila* ATCC 9341 and *Bacillus subtilis* ATCC 6633, respectively (2, 4). Standards were prepared fresh on the day of use in pooled rabbit serum for determinations of serum daptomycin levels and in 5% rabbit serum for determinations of CSF daptomycin levels (4). Assay curves were produced using standard dilutions, including 0.5, 1, 2, 4, 8, 16, 32, and 64 mg/liter daptomycin and vancomycin. For each test, including control rabbit serum samples, a concentration of 20 mg/liter daptomycin or vancomycin was used (4, 6). The sensitivity of the assay was 1 mg/liter for daptomycin and 2 mg/liter for vancomycin. The assay had good reproducibility (±10%).

Statistical analysis. Data were evaluated by using the SPSS version 13.0 program package with the Mann-Whitney U test, Kruskal-Wallis test, and Fisher's χ^2 test. A *P* value of less than 0.05 was considered significant.

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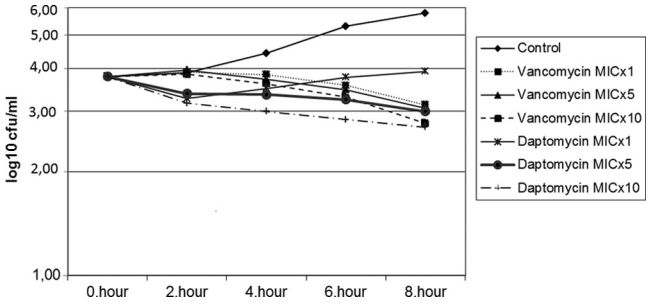

FIG 1 Time-kill results for daptomycin and vancomycin against the study strain.

Ethics. The study was approved by the local ethical committee on animal studies (approval number 2010-15).

***In vitro* killing assays.** The study strain ATCC 43300 was grown in sterile human serum to an optical density of 0.3 at 590 nm and then diluted 4,000-fold to 10^4 CFU, corresponding approximately to bacterial concentrations in the CSF of rabbits before initiation of therapy (4). Daptomycin and vancomycin were added at concentrations corresponding to 1×, 5×, and 10× MIC. Bacterial titers were determined at 0, 2, 4, 6, and 8 h by serial dilution of samples, plated on agar plates containing 5% sheep blood, and incubated at 37°C for 24 h. Experiments were performed in triplicate, and results are expressed as the mean $\log_{10}$ CFU/ml ± the standard deviation.

The antibacterial efficacies of daptomycin and vancomycin against the study strain ATCC 43300 are illustrated in Fig. 1. Neither daptomycin nor vancomycin resulted in killing rates that met the criterion for bactericidal effect (a ≥3-log decrease) or led to sterilization during the 8-h period on the agar plates containing 5× or 10× MIC (Fig. 1). However, with daptomycin, the plates with 5× or 10× MIC resulted in a 1- to 1.5-$\log_{10}$ CFU/ml higher killing rate than on plates containing 1× MIC.

At the beginning of the *in vivo* study, 32 rabbits were inoculated with bacteria, of which 29 were alive and all had developed meningitis at the end of 16-h incubation period. The three rabbits that died during the 16-h incubation period were excluded from further analysis. The remaining 29 animals were separated into three groups: vancomycin, 9 rabbits; daptomycin, 10 rabbits; control groups, 10 rabbits. Mean bacterial concentrations in these three groups were similar (Table 1) (Kruskal-Wallis test, $P > 0.05$). Eight hours after initiation of treatment, all rabbits in both the vancomycin and daptomycin groups showed a partial response, as the bacterial counts decreased significantly in both treatment groups versus the control group (Table 1) (Mann-Whitney U test, $P < 0.05$). However, there was no statistically significant difference between the D and V groups (Mann-Whitney U test, $P > 0.05$).

Daptomycin could be measured in all but one rabbit, which could not be punctured for an adequate CSF sample. The mean CSF daptomycin level was 1.87 ± 0.39 mg/liter, whereas the mean serum level of daptomycin was 57.5 ± 6.2 mg/liter. The CSF/serum daptomycin ratio ranged between 1.9 and 4.1%.

The vancomycin serum level was above the detection limit of the bioassay (2 mg/liter) in just five rabbits, in which the mean vancomycin concentration was 19.2 ± 17.9 mg/liter. The vancomycin CSF level was above the detection limit of the bioassay (2 mg/liter) in just three rabbits. Their vancomycin concentrations were 2, 2, and 3.2 mg/liter, respectively.

During the antibiotic treatment period of 8 h, mortality was similar between the treatment and control groups (0/9 in the vancomycin group, 1/10 in the daptomycin group, and 1/10 in the control group; Fisher's χ^2 test, $P > 0.05$).

MRSA meningitis occurs mostly after central nervous system operations. Treatment is challenging. The first-line treatment for MRSA meningitis is vancomycin (7, 8). Although vancomycin does not penetrate at all to the CSF without inflammation of the meninges, it penetrates to the CSF in a limited amount during meningitis (2, 7, 8). There may be treatment failure in strains with MICs that are >1 mg/dl, which theoretically may also be associated with a 20-mg/kg vancomycin dosage every 12 h (9). Other treatment modalities are teicoplanin, linezolid, vancomycin combined with rifampin, and intrathecal vancomycin (1, 7–9).

Daptomycin is a cyclic lipopeptide with rapid, concentration-dependent bactericidal activity without cell lysis. It is highly effective against Gram-positive multidrug-resistant bacteria. The published experience regarding daptomycin for the treatment of meningitis is only anecdotal (1). There are also limited data related to the pharmacokinetics of daptomycin in the experimental rabbit meningitis model. Gerber et al. (4) compared the efficacy of daptomycin with vancomycin in the experimental rabbit methicillin-sensitive *S. aureus* (MSSA) meningitis model. The dosages led to serum drug levels in concordance with those for humans receiving standard dosages. Daptomycin was given as a single dose of 15 mg/kg, and vancomycin was administered at 20 mg/kg at 0 and 4 hours. After 8 h of daptomycin treatment, the bacterial count decreased 4.54 ± 1.12 $\log_{10}$ CFU/ml, whereas vancomycin led to decrease of 3.43 ± 1.17 $\log_{10}$ CFU/ml ($P < 0.05$). Gerber and colleagues concluded that daptomycin was more effective than vancomycin in the treatment of MSSA meningitis in rabbits. In our study, although daptomycin was administered at the same 15-mg/kg single dose, at 8 hour after drug treatment the bacterial count decreased 3.610 ± 0.677 $\log_{10}$ CFU/ml. Hence, in the MRSA meningitis model, daptomycin decreased the bacterial count around 1 log less than in the MSSA model. In the present study, our time-kill assay also resulted in a steeper curve for daptomycin, with no sterility on the plate during the 8-h treatment period, compared with the results of Gerber et al. The differences in results may be due to different intrinsic properties of the strains and/or bacterial strain virulence. Another explanation may be the higher starting inoculum of approximately 1-$\log_{10}$ CFU/ml in the CSF during the start of therapy and the time-kill assay.

In the present study, daptomycin was administered at 15 mg/kg, because this amount produced serum and CSF drug levels comparable to those reported to correspond to levels obtained in humans administered a daptomycin dose of 6 mg/kg (4). Vanco-

TABLE 1 Cerebrospinal bacterial culture results

Treatment	No. of bacteria ($\log_{10}$ CFU/ml)[a] after:		
	0 h	8 h	Difference
Control	3.448 ± 0.318	4.030 ± 0.495	3.798 ± 0.682
Daptomycin	3.958 ± 0.568	3.371 ± 0.698	−3.610 ± 0.677
Vancomycin	3.703 ± 0.666	3.167 ± 0.760	−3.403 ± 0.697

[a] Data are means ± standard deviations.

mycin, which is a time-dependent bactericidal agent, was given at 0 and 4 h, according to its pharmacokinetic properties described in previous studies (4, 10).

In our experimental study, a bioassay method was used instead of high-performance liquid chromatography, due to cost issues. However, the daptomycin level was above the level of detection in all CSF samples in the bioassay. Even though we could have checked only the trough levels, the trough CSF daptomycin levels were above the MIC. For daptomycin, the bioassay method seems sufficient to detect drug levels in the CSF and blood. Unlike daptomycin, vancomycin levels could not be measured in all the rabbits. Hence, four and six rabbits had lower vancomycin levels in the serum and CSF, respectively, than the lowest detection limit for the bioassay (2 mg/liter). Gerber et al. (4) reported trough CSF vancomycin levels of 1.9 mg/liter after 8 h when they used the same vancomycin dosage regimen as the present study. Our findings are also in concordance with their results.

The relatively short time of observation, the bolus administration of vancomycin rather than a continuous infusion, the absence of peak drug concentrations, and the usage of a bioassay to detect drug levels, which led to detection of vancomycin levels in only a few rabbits, are limitations of the present study.

The first treatment option for MRSA meningitis is vancomycin. To our knowledge, no clinical or experimental study has compared the efficacies of vancomycin and daptomycin for MRSA meningitis; this is the first study to compare the daptomycin and vancomycin efficacies. Our results suggest that daptomycin is at least as effective as vancomycin in the treatment of MRSA meningitis, based on an experimental meningitis model in rabbits. Additional data are necessary to confirm our experiments in advance of clinical trials to assess the efficacy of daptomycin in humans.

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