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Daptomycin is more efficacious than vancomycin against a methicillin-susceptible *Staphylococcus aureus* in experimental meningitis

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Objectives: To test the efficacy of daptomycin, a cyclic lipopeptide antibiotic, against a methicillin-susceptible *Staphylococcus aureus* strain in experimental rabbit meningitis and to determine its penetration into non-inflamed and inflamed meninges

Results: Over a treatment period of 8 h, daptomycin (15 mg/kg) was significantly superior to the comparator regimen vancomycin (–4.54 ± 1.12 $\log_{10}$/mL for daptomycin versus –3.43 ± 1.17 $\log_{10}$/mL for vancomycin). Daptomycin managed to sterilize 6 out of 10 CSFs compared with 4 out of 10 for vancomycin. The penetration of daptomycin into inflamed meninges was ∼5% and ∼2% into non-inflamed meninges.

Conclusions: The superior bactericidal activity of daptomycin was confirmed *in vivo* and in time-killing assays *in vitro*.

Keywords: bacterial meningitis, treatment, *S. aureus*, cyclic lipopeptide

Introduction

Staphylococcus aureus is the cause of 1–9% of all cases of bacterial meningitis with mortality rates ranging from 14 to 77%.[1–3] Usually, meningitis due to *S. aureus* occurs in patients after neurosurgical procedures, head trauma or in those with CSF shunts (12–19% of the cases). In patients without prior central nervous system disease, underlying conditions include diabetes mellitus, alcoholism, haemodialysis, intravenous drug abuse and malignancies. In an epidemiological study, Schlesinger *et al.* reported that 55% of the cases were observed in patients with a variety of central nervous disorders, i.e. CSF shunts, stroke, trauma, haemorrhage, seizure disorders, neoplasm, hydrocephalus and arteriovenous malformations.[3] Other underlying conditions in patients with community-acquired *S. aureus* meningitis include sinusitis, endocarditis, abscess, cellulitis, osteomyelitis and pneumonia. Mortality in patients with *S. aureus* meningitis has been reported to be higher when the way of infection is haematogenous rather than post-operative (56% versus 18%).[2] In patients with CSF shunts, *Staphylococcus epidermidis* is the most common cause of meningitis, accounting for 47–64% of the cases.[4]

Daptomycin is a new lipopeptide antibiotic with an excellent bactericidal activity against Gram-positive microorganisms, including methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant staphylococci.[5–7] We have recently shown that daptomycin was very efficacious in experimental pneumococcal meningitis, sterilizing the CSF of rabbits within 4 h.[8] The aim of this study was to test the efficacy of daptomycin against a methicillin-susceptible staphylococcal strain in the meningitis rabbit model.

Material and methods

Staphylococcal strain

The staphylococcal strain MSSA 1112 was kindly provided by Dr José Entenza, Department of Infectious Diseases, University Hospital Lausanne and Professor Philippe Moreillon, Department of Experimental Microbiology, University of Lausanne, Switzerland. This strain has been routinely used in experimental endocarditis.[9] The strain was grown in Mueller-Hinton broth (MHB) until an approximate density of 10^8 cfu/mL and was then diluted for both *in vitro* and *in vivo* experiments.

Experimental meningitis model

The experimental rabbit meningitis model described by Dacey and Sande[10] was used in this project. The experimental protocols were approved by the Kantonales Veterinäramt des Kantons Bern.

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Pathogen-free New Zealand rabbits were provided by the Zentral-tierställe der Medizinischen Fakultät der Universität Bern, where all the experiments have been performed.

One day before an experiment, rabbits were anaesthetized by intramuscular injection of a combination of ketamine and xylazine to fit prostheses on their calvarium to facilitate subsequent placement within a stereotactic frame. On the day of the experiment, rabbits received 1.75 g/kg ethylcarbamate (urethane) subcutaneously and then 10 mg/kg pentobarbital intravenously to induce deep anaesthesia. The animals were fixed in stereotactic frames and a 3.5 inch (25G) spinal needle was introduced into the cisterna magna. Following the withdrawal of 0.2 mL of CSF, staphylococci (1×10^5 cfu in 0.2 mL of saline solution) were injected into the subarachnoid space. After inoculation the animals were brought back to the cages for the night. About 8 h later, the rabbits were fitted again in the frames using the techniques and anaesthesia described above. A catheter was fixed in the femoral artery for serum sampling. A spinal needle was fixed again in the subarachnoid space. Antibiotics were injected intravenously in doses described in the literature[11,12] (vancomycin 20 mg/kg). Vancomycin doses were standard doses. Daptomycin was injected once (15 mg/kg) at 0 h.

Vancomycin was given at 0 and 4 h, according to its pharmacokinetic properties. CSF (0.2 mL) was sampled at 0, 1, 2, 4, 6 and 8 h after initiation of therapy. Blood samples were collected at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 h after initiation of therapy. Each group included untreated controls, daptomycin and vancomycin treated rabbits, randomly chosen. Experiments were repeated until 10 rabbits were reached in each group. Killing rates were evaluated by linear regression analysis, as described previously.[13] Results were expressed as $\Delta \log_{10}$ cfu/mL·h and $\Delta \log_{10}$ cfu/mL·8 h. For the determination of the penetration of daptomycin into non-inflamed meninges, blood and CSF were sampled just after fixing the rabbits in the frame, without instillation of bacteria into the CSF space.

In vitro killing assays

The staphylococcal strain MSSA 1112 was grown in MHB supplemented with calcium (50 mg/L) to an optical density of 0.3 at 590 nm and then diluted 40-fold to 10^6 cfu corresponding approximately to bacterial concentrations in the CSF of rabbits before initiation of therapy. Daptomycin and vancomycin were added at concentrations corresponding to 1×, 5× and 10× MIC. Bacterial titres 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 mean $\log_{10}$ cfu/mL ± SD.

Determination of antibiotic levels and cfu titres

The concentration of daptomycin in serum and CSF was determined by HPLC. This analytical work was kindly performed by Dr Changfu Chen, Cubist Pharmaceuticals, Lexington. Samples were kept frozen at –80°C and sent on dry ice. The limit of detection was 0.01 mg/L.

The concentration of vancomycin in the CSF was determined by the agar diffusion method. Standard curves were performed in saline with 5% rabbit serum in order to mimic CSF protein concentration.[14] *Bacillus subtilis* ATCC 6633 was used as a test strain.[15] The intra-day and inter-day variation of this method was <10% and the limit of detection of vancomycin was 0.5 mg/L.

Colony forming units were measured by serial dilution of CSF plated on agar plates with 5% sheep blood and incubated overnight at 37°C.
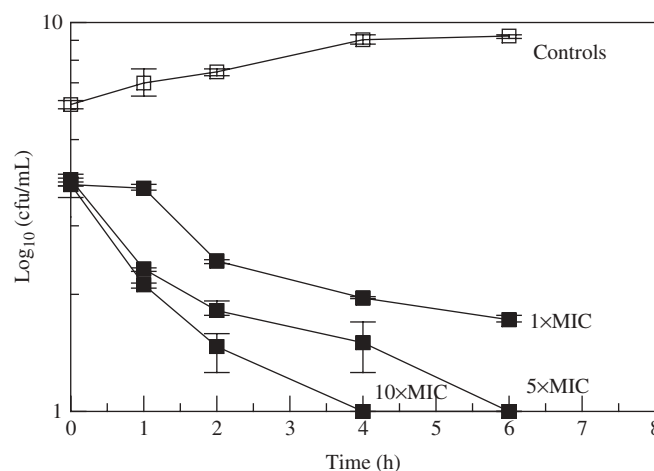

Figure 1. Daptomycin against MSSA *in vitro*. Killing rates of daptomycin (filled squares) *in vitro* at concentrations corresponding to 1×, 5× and 10× MIC against a methicillin-susceptible strain. Open squares, untreated controls. Experiments were performed in triplicate and killing rates are expressed as means ± SD.

Statistical analysis

The Student's *t*-test and one-way analysis of variance (Newman-Keuls multiple comparisons test) were used for parametric data. A *P* value of <0.05 was considered significant.

Results

The antibacterial efficacy of daptomycin against a methicillin-susceptible strain is demonstrated in Figure 1. Even when a concentration around the MIC (1 mg/L) was used, daptomycin was bactericidal with a decrease in viable cell count of ~3.6 $\log_{10}$ after 6 h. At higher concentrations (5 × and 10 × MIC), daptomycin managed to sterilize the cultures after 6 and 4 h, respectively. The comparator regimen (vancomycin) was less bactericidal *in vitro*. 1 × MIC (1 mg/L) of vancomycin produced a decrease in cfu of ~2.3 $\log_{10}$. 5× and 10× MIC were slightly superior with antibacterial activities of 3.2 and 3.4 $\log_{10}$, respectively (Figure 2).

One dose of daptomycin (15 mg/kg) produced a peak serum level of 141 ± 4 mg/L decreasing progressively to 75 ± 16 mg/L at the end of the experimental period. In the CSF, daptomycin peaked at 4.5 mg/L after 2 h, remaining at ~4 mg/L during the entire treatment period and produced CSF/MIC ratios of ~4 during the experimental period (Figure 3, filled squares). The penetration of daptomycin into inflamed meninges was 5%, determined by comparison of AUC serum/CSF. In non-infected animals (Figure 3, open squares) daptomycin levels increased slowly and peaked at ~2.9 mg/L 2 h after injection and remained approximately stable during the rest of the experimental period, leading to a penetration of 2% into non-inflamed meninges. Two injections of vancomycin (20 mg/kg) produced peak and trough levels between 4.0 and 1.9 and 4.5 and 2.2 mg/L respectively (data not shown).

The killing rates of the different regimens, calculated by linear regression analysis, are shown in Table 1. Before starting a therapy, the initial bacterial titre was similar in all treatment groups. In untreated controls, bacterial titres showed minimal growth rates, <1 $\log_{10}$ over 8 h (+0.86 ± 0.36 $\log_{10}$/mL).
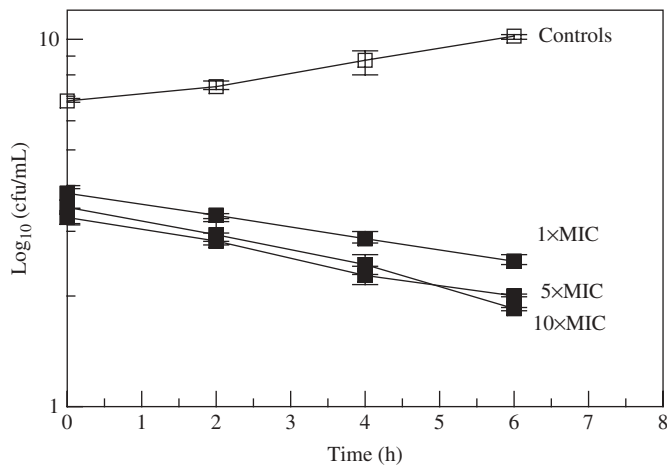
Figure 2. Vancomyin against MSSA *in vitro*. Killing rates of vancomycin (filled squares) *in vitro* at concentrations corresponding to 1×, 5× and 10× MIC against a methicillin-susceptible strain. Open squares, untreated controls. Experiments were performed in triplicate and killing rates are expressed as means ± SD.
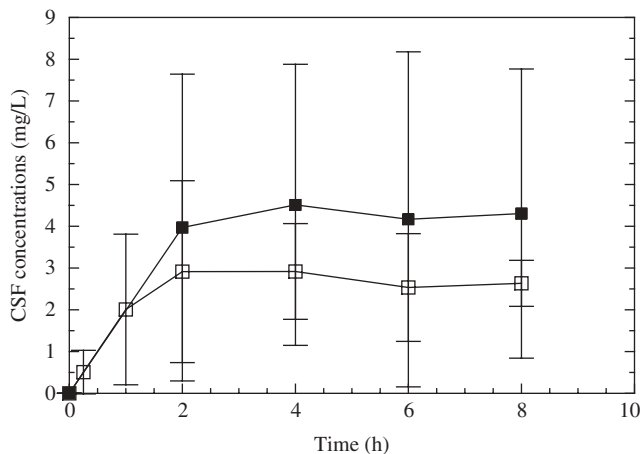

Figure 3. CSF levels of daptomycin (1 × 15 mg/kg) in non-inflamed and inflamed meninges. Daptomycin penetration into the CSF through inflamed (filled squares) and through non-inflamed meninges (open squares) during 8 h after intravenous injection of 15 mg/kg.

Table 1. Daptomycin monotherapy compared with vancomycin against a methicillin-susceptible *S. aureus* (MSSA) in experimental meningitis

Groups (n)	Inoculum ($\log_{10}$ cfu/mL)	Killing rates/h in CSF ($\Delta\log_{10}$ cfu/ mL·h)	Killing rates/ 8 h in CSF ($\Delta\log_{10}$ cfu/ mL·8 h)
Controls (10)	5.09 ± 0.32	+0.11 ± 0.05	+0.86 ± 0.36
Daptomycin (10)	5.50 ± 0.36	–0.59 ± 0.14[a]	–4.54 ± 1.12[b]
Vancomycin (10)	5.18 ± 0.32	–0.43 ± 0.24[a]	–3.43 ± 1.17[b]

Results are expressed as means ± SD.
[a]Daptomycin versus vancomycin not significant.
[b]$P < 0.04$ for daptomycin versus vancomycin.

Daptomycin produced a good bactericidal activity against the methicillin-susceptible staphylococcal strain, leading to a decrease of 4.54 $\log_{10}$ cfu/mL over 8 h. Daptomycin managed to sterilize 6 out of 10 CSFs of rabbits. Two doses of vancomycin sterilized 4 out of 10 CSFs of rabbits and were significantly less efficacious than daptomycin (–3.43 ± 1.17 $\log_{10}$ versus –4.54 ± 1.12 $\log_{10}$ for daptomycin; *P*<0.04).

Discussion

Meningitis due to *S. aureus*, occurring mostly after head trauma or neurosurgical interventions, remains one of the most deleterious infections of the central nervous system. In case of β-lactam allergy, and when methicillin-resistant strains are suspected, vancomycin is the treatment of choice. However, the unreliable penetration for vancomycin into the CSF, especially with addition of dexamethasone, remains a matter of concern,[11,16] underlining the need of alternative therapies. Daptomycin is a new cyclic lipopeptide antibiotic with an excellent activity against a variety of Gram-positive microorganisms, including staphylococci.[17,18] We have recently shown that daptomycin was very efficacious against a penicillin-resistant and a penicillin- and quinolone-resistant pneumococcal strain in experimental meningitis. The most interesting feature of that study was the fact that daptomycin managed to sterilize the CSFs of rabbits within 4 h.[8]

In this study daptomycin (15 mg/kg) produced serum and CSF levels comparable to those described previously in pneumococcal meningitis[8] and corresponded to levels obtained in humans with a dose of 6 mg/kg. We are aware that nafcillin or flucloxacillin is the standard treatment of MSSA meningitis in humans. However, because of their pronounced side effect i.e. profuse diarrhoea, we decided to use vancomycin as the comparator treatment in this study. The dose of vancomycin (2 × 20 mg/kg) was a standard dose used in previous studies[19,20] and produced CSF levels similar to those measured in humans with high-dose therapy.[21,22] The CSF levels of both treatment groups were roughly equivalent with analogous CSF/MIC ratios. The superiority of daptomycin is probably due to its more pronounced bactericidal activity against staphylococci as demonstrated in time-killing assays *in vitro* (Figures 1 and 2). In the same experimental meningitis model, daptomycin was less efficacious against a staphylococcal strain than against pneumococci probably owing to the different MICs (0.06 mg/L for the pneumococcal strains versus 1 mg/L for the methicillin-susceptible staphylococcal strain). The degree of inflammation of the meninges also influences the penetration of daptomycin into the CSF (2% into non-inflamed versus 5% into inflamed meninges), but to a lesser extent compared with other antibiotics, e.g. β-lactams, probably owing to its lipophilic properties.

In summary, the rapid bactericidal activity and the pronounced efficacy of daptomycin against a methicillin-susceptible staphylococcal strain designate daptomycin as an alternative treatment for staphylococcal infections of the CNS and confirmed its efficacy against Gram-positive microorganisms observed in other animal models.[6]

Acknowledgements

Transparency declarations

None to declare.

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