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Efficacy of dalbavancin in the treatment of MRSA rat sternal osteomyelitis with mediastinitis

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Objectives: Dalbavancin, a semi-synthetic lipoglycopeptide, is characterized by a long plasma half-life, which allows weekly dosing. Dalbavancin may be a good treatment option for patients with deep sternal wound infections owing to its improved pharmacokinetic profile and antibacterial activity compared with currently used antibiotics. Here we evaluated the efficacy of 7 or 14 days of treatment with dalbavancin, compared with vancomycin and with saline, in reducing sternal bone MRSA counts in a rat *Staphylococcus aureus* deep sternal wound infection model.

Methods: A mid-sternal wound was surgically induced in anaesthetized rats. A clinical strain of MRSA was injected into the sternum to establish infection. Rats were treated intraperitoneally for 7 or 14 days with dalbavancin, vancomycin or saline. The number of cfu per gram of sternum or spleen tissue was determined using viable counts. The antibacterial efficacy was determined by the reduction in bacterial counts per gram of sternum or spleen tissue in each treatment group.

Results: Treatment with dalbavancin was superior to treatment with saline for 7 days (0.75 log reduction in bone cfu) or 14 days (>3 log reduction in bone cfu) and similar to treatment with vancomycin. Additionally, dalbavancin was also effective in reducing systemic dissemination of MRSA.

Conclusions: Dalbavancin is effective in the treatment of MRSA rat sternal osteomyelitis.

Introduction

Deep sternal wound infection and mediastinitis (DSWI) is reported to occur in 1%–4% of patients undergoing cardiac surgery.[1] DSWI results in severe clinical and economic consequences, such as additional hospital days and an increased cost of care.[2,3]

Staphylococcus aureus and *Staphylococcus epidermidis* are the most common pathogens associated with DSWI, causing about 34%–54% and 12%–44% of cases, respectively.[4–9] Organisms isolated from DSWI are often methicillin-resistant.[9–11] Glycopeptide antibiotics such as vancomycin and teicoplanin are commonly used to treat these infections. These agents require prolonged intravenous access and show difficulty in maintaining tissue drug levels above the MICs. Therefore, new treatment modalities that improve outcomes and convenience while reducing the duration of hospitalization and its associated costs are needed.

Dalbavancin is a semi-synthetic lipoglycopeptide antibiotic agent with a long plasma half-life, which allows weekly dosing. It has greater potency against many Gram-positive organisms compared with teicoplanin and vancomycin[12,13] and has been recently approved by the US FDA for the treatment of acute bacterial skin and skin-structure infections in adults. Therefore, dalbavancin may be a good treatment option for patients with deep sternal wound infections due to its improved pharmacokinetic (PK) profile and antibacterial activity.

Here, we evaluated the efficacy of dalbavancin versus vancomycin in reducing sternal bone bacterial counts in a rat MRSA deep sternal wound infection model. Previous studies have demonstrated that this model results in 100% osteomyelitis and sternal abscess, which allows quantitative measurement of bacterial counts in the sternum as well as assessment of systemic dissemination of the infection.[14,15]

Methods

Median sternotomy model

Male Lewis rats, 10 weeks of age, weighing 250–270 g (Harlan Laboratories, Rehovot, Israel) were housed two rats per cage in a central animal research facility. Conditions and care as well as the median sternotomy model were previously described.[14,15] All procedures, care and handling of the animals were reviewed and approved by the Institutional Animal Care and Use Committee at the Tel-Aviv Sourasky Medical Center.

Bacterial strain and infection

A clinical isolate of *S. aureus* (MRSA; strain 3020) from the sternal bone of a patient with DSWI was used in all experiments. The isolate MIC of vancomycin was 1 mg/L and the isolate MIC of dalbavancin was 0.06 mg/L, using both agar dilution and broth dilution. Infecting inoculum was freshly prepared before the experiment, and was confirmed by quantitative cultures. Infection was induced after mid-sternal incision by precise injection of 0.05 mL (1×10^7 cfu/rat) of MRSA into the exposed sternal edges and the spongiosa. The muscle layer and skin were sutured in layers with 4-0 nylon monofilaments. The animals' survival and the chest wounds were assessed daily.

Lewis rats ($n=84$) were randomly divided into three groups treated with one of the following regimens: (i) dalbavancin, given intraperitoneally, 20 mg/kg loading dose followed by 10 mg/kg daily; (ii) vancomycin, given intraperitoneally, 50 mg/kg, every 12 h; and (iii) saline control group, 0.9% NaCl, given intraperitoneally, once daily. Treatment started 24 h after the induction of infection and continued for 7 or 14 days.

Rats were sacrificed 24 h after the last treatment dose was given. Sternal bone and spleen were harvested. Specimens were homogenized, and the number of cfu/g sternum or spleen tissue was determined on blood agar plates.

Bone level assays

A piece of the femur was removed from rats sacrificed on days 4 ($n=10$), 6 ($n=4$) and 10 ($n=4$); sternal bone was also removed from rats on day 4. Samples were placed in saline and shipped to ICON (Oriskany, NY, USA) for measurement of dalbavancin levels based on assays previously described.[16]

Statistical analysis

The log-transformed cfu/g of sternal bone was compared between the treatment groups. Zero values were replaced by 1 to avoid nullification of multiplicative values.[17] Geometric means and 95% CIs were used for graphical representation, and Student's *t*-test was performed on the log-transformed cfu/g sternal bone to compare differences between the treatment groups, as recommended for skewed data.[18,19] Differences in proportion were tested using the χ^2 test. Analyses were performed using GraphPad Prism version 5 (San Diego, CA, USA) and Stata version 12 (College Station, TX, USA). $P\leq0.05$ was considered statistically significant.

Results

Sternal osteomyelitis was induced by surgical sternotomy and inoculation of the incised sternal bone with MRSA (10^7 cfu/sternum). Eighty-four rats were divided into three treatment groups 1 day after induction of the infection and treated with dalbavancin, vancomycin or placebo (saline). Each group of animals was subdivided into two groups: a 7 day treatment group (three experiments, $n=58$) and a 14 day treatment group (two experiments, $n=26$).

No mortality was observed among the treated rats. At the end of the treatment period, the rats were sacrificed and sternal bone was excised and weighed and cfu per gram of sternal bone was determined in triplicate. In the saline-treated group (control), we detected 8.5×10^6 cfu/g sternum and 2.3×10^6 cfu/g sternum at 7 and 14 days, respectively, and 100% osteomyelitis, similar to previous results with this model.[14]

Comparison of the treatment groups

Dalbavancin at doses simulating a human dose of 1 g led to a significant reduction in sternal bone MRSA cfu count, compared with saline, after 7 days of treatment (geometric mean cfu/g sternum $=1.5\times10^5$ versus 2.16×10^6 cfu/g sternum, respectively, $P=0.0011$) and after 14 days of treatment (geometric mean cfu $=2.6\times10^2$ versus 6.2×10^5, respectively, $P=0.006$). The treatment effect of dalbavancin was similar to that of vancomycin both at 7 days (geometric mean $=4.1\times10^4$, $P=0.35$) and at 14 days (geometric mean $=5.3\times10^1$, $P=0.53$). Results comparing the geometric means and 95% CIs are presented in Figure 1.

Systemic dissemination

Quantitative bacterial counts from the spleen were determined in rats treated for 7 days. Six of the 18 saline-treated rats had systemic dissemination, as evident by bacterial growth from the extracted organs, while only 1/20 rats treated with dalbavancin or vancomycin revealed systemic dissemination of infection ($P=0.025$).

Dalbavancin led to a statistically significant decrease in the proportion of rats with systemic dissemination, compared with treatment with saline, after 7 days of treatment (5% and 33%, respectively, with systemic dissemination, $P=0.025$). The treatment effect of dalbavancin was similar to that achieved by vancomycin ($P=1$). Percentage body weight lost at day 6 was similar in the vancomycin and dalbavancin groups ($5.1\pm2.6\%$ and $4.1\pm2.7\%$, respectively). However, at day 11 a higher percentage of body weight was lost in the vancomycin-treated group than in the dalbavancin-treated group ($4.9\pm3.2\%$ versus $1.9\pm3.5\%$, $P=0.03$).

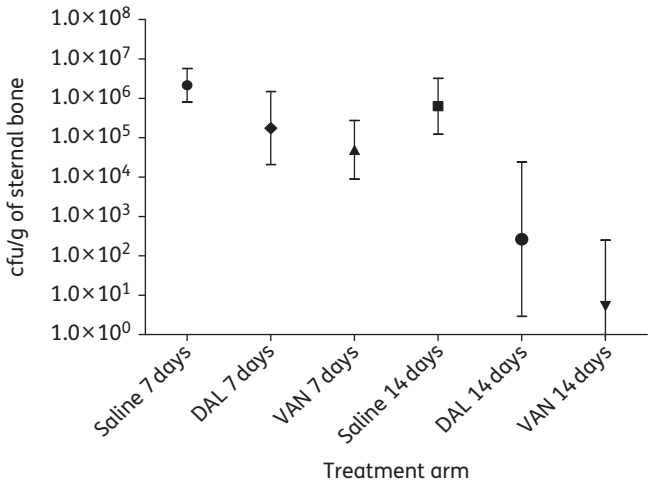

Figure 1. Geometric means and 95% CIs of *S. aureus* sternal bone cfu counts. VAN, vancomycin; DAL, dalbavancin.

Table 1. Concentrations of dalbavancin[a] in sternal and femoral bones in rats

Sample	Number of samples	Days of dosing	Concentration, μg/g	Range, μg/g (SD)
Sternum	10	4	9.5	5.7–12.6 (2.7)
Femur	10	4	7.5	4.4–12.4 (2.6)
	4	6	9.2	6.8–10.9 (1.8)
	4	10	10.7	8.3–15.9 (3.5)

[a]Rats were dosed with dalbavancin intraperitoneally (20 mg/kg on day 1 followed by 10 mg/kg per day).

Bone dalbavancin level

Dalbavancin concentrations in the sternum after 4 days of daily dosing were 9.5 μg/g and in the femur were 7.5 μg/g. Levels in the femur on days 6 and 10 were 9.2 and 10.7 μg/g, respectively (Table 1).

Discussion

Dalbavancin distributes into bone and associated tissue, including synovium. In humans after a single intravenous 1000 mg dose, dalbavancin bone levels were 4.6 μg/g at day 3 and remained at 4.1 μg/g on day 14.[16] Based on a comparison with serum levels, the bone penetration was 13.9%. The high degree of protein binding (93%) suggests that the overall penetration of dalbavancin into bones is high relative to free-drug plasma concentrations and supports further study in patients with bone and joint infections. Dalbavancin may be a good treatment option for patients with deep sternal wound infections due to its improved PK profile and antibacterial activity, and bone penetration compared with currently used antibiotics.

In this study, dalbavancin and vancomycin were given at doses expected to provide exposures similar to those seen in humans with the approved dosing regimens. Dalbavancin levels in bone measured at days 4 and 10 in the femur of these rats were 7.5 and 10.7 μg/g, respectively, similar to levels seen in humans after a single 1 g dose. Levels of dalbavancin in the sternum on day 4 were 9.5 μg/g, demonstrating effective penetration of dalbavancin into the infected sternal bone. As far as we are aware, this is the first demonstration that the efficacy of dalbavancin is similar to that of vancomycin for the treatment of sternal osteomyelitis caused by MRSA and is superior to placebo therapy. Dalbavancin treatment was also associated with a statistically significant reduction in systemic dissemination of the MRSA infection when compared with placebo therapy.

There were a few limitations of this study. The duration of therapy utilized in this study was shorter than the duration of 28 days that is generally used in animal models of MRSA sternal osteomyelitis.[20] This may have led to an under-estimation of the efficacy of dalbavancin. The study used a single isolate of MRSA. Results with other strains may vary.

Conclusions

This study demonstrated the effectiveness of dalbavancin using doses mimicking human PK following a single intravenous dose of 1000 mg in the treatment of MRSA rat sternal osteomyelitis. Treatment with dalbavancin was superior to treatment with saline for 7 or 14 days (0.75 and >3 log reduction in bone cfu, respectively). Treatment with dalbavancin for 7 days reduced systemic dissemination of MRSA compared with treatment with saline (5% versus 33%). Results were similar to those achieved by treatment with vancomycin.

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Transparency declarations

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