High-Dose Daptomycin Is Effective as an Antibiotic Lock Therapy in a Rabbit Model of *Staphylococcus epidermidis* Catheter-Related Infection

Jana Basas,^a Marta Palau,^a Carlos Ratia,^a José L. del Pozo,^b María Teresa Martín-Gómez,^c Xavier Gomis,^a Eduard Torrents,^d Benito Almirante,^a Joan Gavalda^a

^aAntimicrobial Resistance Laboratory, Vall d'Hebron Research Institute (VHIR), Infectious Diseases Department, Hospital Universitari Vall d'Hebron, Barcelona, Spain

^bInfectious Disease Division, Internal Medicine Department, Clínica Universitaria de Navarra, Navarra, Spain

^cMicrobiology Department, Hospital Universitari Vall d'Hebron, Barcelona, Spain

^dInstitute for Bioengineering of Catalonia (IBEC), Bacterial Infections and Antimicrobial Therapies, Barcelona, Spain

ABSTRACT Long-term catheter-related bloodstream infections (CRBSIs) involving coagulase-negative staphylococci are associated with poor patient outcomes, increased hospitalization, and high treatment costs. The use of vancomycin lock therapy has been an important step forward in treatment of these biofilms, although failures occur in 20% of patients. In this study, we report that a high dose of daptomycin lock therapy may offer a therapeutic advantage for these CRBSIs in just 24 h of treatment.

KEYWORDS catheter-related infection, daptomycin, animal model, antibiotic lock therapy, coagulase-negative staphylococci, *S. epidermidis*

Bloodstream infections (BSIs) are a common complication of central venous catheters (CVCs), with incidence rates of 2.9 to 11.3 cases per 1,000 days of CVC use (1). The main microorganisms that cause long-term catheter-related BSIs (CRBSIs) are coagulase-negative staphylococci (CoNS), which account for ~30% of health care-associated BSIs (2–5). The pathogenic property of CoNS is related mainly to their ability to form an adherent biofilm on the surface of indwelling medical devices (6). Antibiotic lock therapy (ALT) using vancomycin lock therapy (LT) is recommended in cases of uncomplicated CRBSIs caused by CoNS (7). Moreover, this treatment fails in the form of relapse in 20% of episodes, so new therapeutic alternatives need to be explored as elective therapies (2). Daptomycin has demonstrated high *in vitro* efficacy against staphylococcal biofilms because of rapid penetration into the matrix of the biofilm (8, 9).

ALT regimens last 14 days, resulting in reduced access to the device. Moreover, there are patients with no other available vascular access who urgently need vascular access and benefit from a shorter treatment period. Given the clinical importance of finding a faster and more effective ALT treatment to enable earlier access to the long-term catheter, thereby improving patient outcomes, reducing the risk of resistance, and preventing infection-related sequelae, the aim of this study was to compare the activity of vancomycin and different concentrations of daptomycin for the treatment of experimental *Staphylococcus epidermidis* catheter-related infection using ALT in a rabbit model.

The MICs of vancomycin and daptomycin against two strains of *S. epidermidis* (SE14 and SE94) were determined with the broth microdilution method according to EUCAST (10). Both strains were susceptible to vancomycin and daptomycin. To analyze the susceptibility on biofilm formation, we followed the protocol described by Chandra et al. (11). We found that minimum biofilm eradication concentrations at 90% (MBEC_{90s})

Received 28 August 2017 Returned for modification 9 October 2017 Accepted 11 November 2017

Accepted manuscript posted online 20 November 2017

Citation Basas J, Palau M, Ratia C, del Pozo JL, Martín-Gómez MT, Gomis X, Torrents E, Almirante B, Gavalda J. 2018. High-dose daptomycin is effective as an antibiotic lock therapy in a rabbit model of *Staphylococcus epidermidis* catheter-related infection. Antimicrob Agents Chemother 62:e01777-17. <https://doi.org/10.1128/AAC.01777-17>.

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Address correspondence to Joan Gavalda, joan.gavalda@vhir.org.

B.A. and J.G. contributed equally to this work.

FIG 1 Activity of vancomycin and daptomycin in silicone discs against *S. epidermidis* strains SE14 (a) and SE94 (b) after 24 h of treatment. VAN, vancomycin; DAP, daptomycin. *, $P \leq 0.05$ versus control. **, $P \leq 0.05$ versus control and VAN 10 mg/ml. ***, $P \leq 0.05$ versus control, VAN 10 mg/ml, and DAP 5 mg/ml. (c) *S. epidermidis* stained with LIVE/DEAD BacLight viability kit to directly visualize the effect of the antibiotics tested. Red fluorescence, dead cells; green fluorescence, live cells (visualized at $\times 60$ magnification).

of vancomycin for the SE14 and SE94 biofilms growing in silicone discs were 10,000 and $>10,000$ mg/liter, respectively, and the MBEC₉₀s of daptomycin were 1,024 mg/liter for both strains. Our *in vitro* studies indicated that daptomycin exhibited better *in vitro* activity than vancomycin against the *S. epidermidis* biofilm. Unlike daptomycin, vancomycin diffused slowly into the deeper layers of the biofilm, and the biofilm bacteria showed decreased sensitivity to vancomycin (12).

To measure the effect of vancomycin 10 mg/ml and daptomycin 5 and 50 mg/ml on *S. epidermidis* biofilm, growth on silicone discs was visualized with confocal laser scanning microscopy (CLSM) using LIVE/DEAD staining to distinguish between dead cells and live cells. As shown in Fig. 1a and b, daptomycin 50 mg/ml significantly reduced the number of live cells; in contrast, dead cells increased in the other treatments. Moreover, the effect of the treatments visualized with CLSM revealed that both strains treated with daptomycin at a high concentration had a bacterial mortality of $>90\%$ (Fig. 1c). The differences between the results of colony counts and viability using CLSM indicated that some samples contained viable but nonculturable (VBNC) cells. The possible recurrence of staphylococci infections related to medical devices may be caused for this VBNC (13).

Following methodology used in our previous studies of ALT therapy for catheter-related infection (14), after 48 h infection with an inoculum of 10^8 CFU/ml of SE14 or SE94, treatments with vancomycin 10 mg/ml and daptomycin 5 and 50 mg/ml were analyzed using the percentage of negative cultures and the log₁₀ CFU (mean $\pm$ SD; 95% confidence interval [CI]) recovered from the catheter-tip cultures (Table 1). Higher

TABLE 1 Antibiotic lock therapy results for *S. epidermidis* SE14 and SE94 at 24 and 72 h

Antibody lock therapy, by strain ^a	Results at indicated follow-up time			
	24 h		72 h	
	Negative/total cultures (%)	Log ₁₀ total CFU (mean ± SD [95% CI])	Negative/total cultures (%)	Log ₁₀ total CFU (mean ± SD [95% CI])
<i>S. epidermidis</i> SE14				
Control	0/10 (0)	6.3 ± 0.6 (5.8–6.7)	0/13 (0)	5.6 ± 1.1 (4.9–6.2)
DAP 50 mg/ml	12/14 (86) ^b	0.1 ± 0.3 (0.0–0.3) ^b	13/14 (93) ^c	0.1 ± 0.3 (0.0–0.3) ^c
DAP 5 mg/ml	0/13 (0)	1.4 ± 0.4 (1.2–1.7) ^c	11/15 (73) ^c	0.3 ± 0.3 (0.1–0.4) ^c
VAN 10 mg/ml	0/10 (0)	3.8 ± 0.9 (3.2–4.5) ^d	2/14 (14)	2.4 ± 1.5 (1.6–3.3) ^d
<i>S. epidermidis</i> SE94				
Control	0/10 (0)	5.5 ± 1.0 (4.8–6.3)	0/12 (0)	5.6 ± 0.5 (5.3–6)
DAP 50 mg/ml	12/13 (92) ^b	0.1 ± 0.2 (0.0–0.2) ^b	11/14 (79) ^c	0.4 ± 1.0 (0.0–1.0) ^c
DAP 5 mg/ml	0/10 (0)	1.7 ± 0.3 (1.4–1.9) ^c	6/11 (55) ^f	1.3 ± 1.6 (0.2–0.4) ^{d,e}
VAN 10 mg/ml	0/10 (0)	4.4 ± 1.0 (3.7–5.1) ^d	1/12 (8)	2.9 ± 1.3 (2.0–3.7) ^d

^aDAP, daptomycin; VAN, vancomycin.^b*P* ≤ 0.001 versus control, VAN 10 mg/ml and DAP 5 mg/ml.^c*P* ≤ 0.001 versus control and VAN 10 mg/ml.^d*P* ≤ 0.001 versus control.^e*P* ≤ 0.05 versus VAN 10 mg/ml.^f*P* ≤ 0.05 versus control.

concentrations of vancomycin were not used because of the precipitation of the drug (15). After 24 h of LT with daptomycin 50 mg/ml, the colony counts recovered from the catheter-tip cultures were significantly lower than those treated with vancomycin or daptomycin 5 mg/ml (*P* ≤ 0.001). Of note, daptomycin 50 mg/ml was the only LT that achieved a significant percentage of negative catheter-tip cultures in both strains after 24 h. At 72 h, no difference was seen between the two daptomycin doses, which were more effective than the vancomycin locks. This suggests that daptomycin at high concentrations rapidly penetrates the biofilm layer, thus reducing both the length and frequency of ALT with a rapid salvage of CVCs.

Clinical use of daptomycin LT for the treatment of CoNS-associated CRBSIs is scarce (16, 17). Therefore, global results from the few clinical cases in which daptomycin 5 mg/ml was used as ALT are similar to those with vancomycin, with a failure rate of close to 20% to 25%. However, in our results, a high therapeutic dose of daptomycin resulted in statistically significant reductions of catheter bacterial loads and negativization of catheter tips in just 24 h of treatment. That difference can be translated to superior efficacy in clinical practice, including a reduction in the duration of treatment or prevention of the spread of persistent cells.

In conclusion, daptomycin LT at high concentrations may be a promising treatment for CoNS-associated CRBSIs, offering a therapeutic advantage over low concentrations of daptomycin or vancomycin, thus warranting further consideration and clinical studies.

ACKNOWLEDGMENTS

This study was financed by the European Regional Development Fund (ERDF), Spanish Network for the Research in Infectious Diseases (REIPI RD12/0015).

We acknowledge the professional manuscript services of Nature Publishing Group, Language Editing.

This work was conducted within the framework of the Doctorate in Medicine from the Universitat Autònoma de Barcelona.

We have no conflicts of interest to declare.

REFERENCES

- O'Grady NP, Alexander M, Dellinger EP, Gerberding JL, Heard SO, Maki DG, Masur H, McCormick RD, Mermel LA, Pearson ML, Raad II, Randolph A, Weinstein RA. 2002. Guidelines for the prevention of intravascular catheter-related infections, U.S. Pediatrics 110:e51.
- Mermel LA, Allon M, Bouza E, Craven DE, Flynn P, O'Grady NP, Raad II, Rijnders BJ, Sherertz RJ, Warren DK. 2009. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis 49:1–45. <https://doi.org/10.1086/599376>.
- Fernandez-Hidalgo N, Almirante B, Calleja R, Ruiz I, Planes AM, Rodriguez

- D, Pigrau C, Pahissa A. 2006. Antibiotic-lock therapy for long-term intravascular catheter-related bacteraemia: results of an open, non-comparative study. *J Antimicrob Chemother* 57:1172–1180. <https://doi.org/10.1093/jac/dkl103>.
4. Rupp ME. 2013. Clinical characteristics of infections in humans due to *Staphylococcus epidermidis*, p 1–16. In Fey PD (ed), *Staphylococcus epidermidis* methods and protocols. Springer, Omaha, NE.
5. Otto M. 2014. *Staphylococcus epidermidis* pathogenesis. *Methods Mol Biol* 1106:17–31. https://doi.org/10.1007/978-1-62703-736-5_2.
6. Mack D, Davies AP, Harris L, Jeeves R, Pascoe B, Knoblock JM, Rohde H, Wilkinson TS. 2013. *Staphylococcus epidermidis* in biomaterial-associated infection. Springer, New York.
7. Hoiby N, Bjarnsholt T, Moser C, Bassi GL, Coenye T, Donelli G, Hall-Stoodley L, Høla V, Imbert C, Kirketerp-Møller K, Lebeaux D, Oliver A, Ullmann AJ, Williams C, ESCMID Study Group for Biofilms and Consulting External Expert Werner Zimmerli. 2015. ESCMID guideline for the diagnosis and treatment of biofilm infections 2014. *Clin Microbiol Infect* 21(Suppl 1):S1–S25.
8. LaPlante KL, Rybak MJ. 2004. Daptomycin—a novel antibiotic against Gram-positive pathogens. *Expert Opin Pharmacother* 5:2321–2331. <https://doi.org/10.1517/14656566.5.11.2321>.
9. Stewart PS, Davison WM, Steenbergen JN. 2009. Daptomycin rapidly penetrates a *Staphylococcus epidermidis* biofilm. *Antimicrob Agents Chemother* 53:3505–3507. <https://doi.org/10.1128/AAC.01728-08>.
10. European Committee on Antimicrobial Susceptibility Testing. 2016. Breakpoint tables for interpretation of MICs and zone diameters, version 6.0. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_6.0_Breakpoint_table.pdf.
11. Chandra J, Mukherjee PK, Ghannoum MA. 2008. In vitro growth and analysis of *Candida* biofilms. *Nat Protoc* 3:1909–1924. <https://doi.org/10.1038/nprot.2008.192>.
12. Mascio CT, Alder JD, Silverman JA. 2007. Bactericidal action of daptomycin against stationary-phase and nondividing *Staphylococcus aureus* cells. *Antimicrob Agents Chemother* 51:4255–4260. <https://doi.org/10.1128/AAC.00824-07>.
13. Zandri G, Pasquaroli S, Vignaroli C, Talevi S, Manso E, Donelli G, Biavasco F. 2012. Detection of viable but non-culturable staphylococci in biofilms from central venous catheters negative on standard microbiological assays. *Clin Microbiol Infect* 18:E259–E261. <https://doi.org/10.1111/j.1469-0691.2012.03893.x>.
14. Meije Y, Almirante B, Del Pozo JL, Martin MT, Fernandez-Hidalgo N, Shan A, Basas J, Pahissa A, Gavalda J. 2014. Daptomycin is effective as antibiotic-lock therapy in a model of *Staphylococcus aureus* catheter-related infection. *J Infect* 68:548–552. <https://doi.org/10.1016/j.jinf.2014.01.001>.
15. Del Pozo JL. 2009. Role of antibiotic lock therapy for the treatment of catheter-related bloodstream infections. *Int J Artif Organs* 32:678–688.
16. Tatarelli P, Parisini A, Del Bono V, Mikulska M, Viscoli C. 2015. Efficacy of daptomycin lock therapy in the treatment of bloodstream infections related to long-term catheter. *Infection* 43:107–109. <https://doi.org/10.1007/s15010-014-0675-4>.
17. Del Pozo JL, Rodil R, Aguinaga A, Yuste JR, Bustos C, Montero A, Espinosa G, García-Fernández N. 2012. Daptomycin lock therapy for gram-positive long-term catheter-related bloodstream infections. *Int J Clin Pract* 66:305–308. <https://doi.org/10.1111/j.1742-1241.2011.02830.x>.