Comparative Activities Of Daptomycin, Linezolid, And Tigecycline Against Catheter-Associated Methicillin-Resistant Staphylococcus Bacteremic Isolates Embedded In Biofilm

Dr. C. Vijai Prasad[1], Dr. M. Abhul Sathar Sait[2], Dr. A. Ponnambalam[1*]

[1]Associate Professor, Department of General Medicine
Sri Venkateshwaraa Medical College Hospital & Research Centre,
Puducherry - 605107

[2]Assistant Professor, Department of General Medicine
Sri Venkateshwaraa Medical College Hospital & Research Centre,
Puducherry - 605107

***Corresponding Author: Dr. A. Ponnambalam,**
Associate Professor, Department of General Medicine, Sri Venkateshwaraa Medical College Hospital & Research Centre,
Villupuram Main Road, Ariyur, Puducherry – 605107.

ABSTRACT

A vascular catheter may be saved if an intraluminal antibiotic lock treatment is used in the case of catheter-related bloodstream infections. These recently developed antibiotics were compared to the older antibiotics vancomycin and minocycline against methicillin-resistant Staphylococcus aureus (MRSA) embedded in biofilms in this in vitro study. We examined the emergence of MRSA after four hours of daily catheter lock therapy that was resistant to these antibiotics alone and with rifampin. Compared to linezolid, vancomycin, and the negative control, minocycline, daptomycin, and tigecycline are more effective at inhibiting MRSA in biofilms after the first day of exposure (P 0.001), with minocycline being the most active and daptomycin and tigecycline having weak activity, similar to the negative control. Among the antibiotics tested, daptomycin was the fastest in eliminating MRSA from biofilm after three days of exposure for 4-hour periods, followed by minocycline and tigecycline, which were faster than linezolid, rifampin, and vancomycin (P 0.001). After five days of exposure to MRSA continuously for four hours each day, rifampin alone was least effective at eradicating MRSA from biofilms because it promoted rifampin-resistant MRSA growth. It has been found that when rifampin is combined with additional antibiotics, the combination is far more effective at eliminating MRSA colonization than any single antibiotic alone. Daptomycin, minocycline, and tigecycline lock therapy also needs to be researched, as should the potential for rifampin's antistaphylococcal activity as a single agent, but not as a combined solution.

KEYWORDS: Vancomycin, Minocycline against methicillin-resistant Staphylococcus aureus (MRSA), Daptomycin.

INTRODUCTION

A catheter-related bloodstream infection (CRBSI) occurs when methicillin-resistant staphylococci colonise the central venous catheter (CVC). Such colonisation occurs when staphylococci attach themselves to the biofilm layer in the catheter lumen. Recently, guidelines recommend antibiotic catheter lock therapy (ALT) as a means of preventing or treating CRBSI in high-risk patients because of the cost, difficulty, and complications associated with the removal of a long-term CVC and the insertion of a new one at a different site (16).

In order to treat intraluminal ALT, there are a number of medications that are active against methicillin-resistant Staphylococcus aureus (MRSA). We suggest using these medications (6, 8, 10, 14, 27). As MRSA organisms bury in biofilm on catheter surfaces and become resistant to glycopeptides, such as vancomycin in suspension, resistance develops (6, 8, 9, 23, 27). By using an in vitro silicone disc biofilm colonization model, we determined if new treatments (daptomycin, linezolid, and tigecycline) could be as effective against catheter-related bacteremic MRSA embedded in biofilm as older therapies (vancomycin, minocycline, and rifampin). Also, we examined the potential for antibiotic resistance to develop under daily catheter lock therapy, either alone or in combination with rifampin.

MATERIALS AND METHODS SYNOPSIS

Antibacterial properties are present in biofilms. A silicone disc biofilm colonisation in vitro model (13), used to test antibiotic efficacy in eradicating MRSA embedded in biofilms, was used to test different antibiotics' efficacy (14). Incubation at 37 degrees Celsius with shaking of silicon discs and human plasma was performed for 24 hours. Inoculating 1 ml of bacterial inoculum into the plasma and shaking it for 24 hours at 37°C inoculated the plasma with bacteria. For making the bacterial inoculum (MHB), 30 MRSA isolates that had produced CRBSI in Mueller-Hinton broth were diluted to 5.5 105 cells/ml. Four different organisms were tested against each drug. As soon as they were removed from the infected broth, the silicone discs were soaked in 0.9% saline wash for 30 minutes.

In the following step, the silicone discs were placed in new tubes containing MHB or 2-mg/ml solutions of the following drugs: I daptomycin (supplemented with 50 mg/liter calcium), II linezolid, III minocycline, IV tigecycline, V rifampin, VI vancomycin. A 24 hour incubation at 37°C was followed by submersion in 0.9 percent saline and subsequent sonication for 15 minutes. After vortexing for 30 seconds, the tubes were incubated overnight at 37°C in Trypticase soy agar with 5% sheep blood and 100 l of saline. A dilution factor was taken into account when calculating the final counts of the colonies..

On a daily basis, antibiotic lock is activated.

We repeated this procedure for 10 MRSA isolates, immersing the colonized discs every day in a drug solution for four hours, followed by incubating the discs for 24 hours at 37°C to test the antibiotics' cyclic short-term applications in a manner that mimics daily antibiotic catheterization. Five days of treatment were required for this treatment. Each silicone disc was treated with antibiotics for 4 hours per day, either alone or together with rifampin. After incubating the silicone discs for four hours, four to six from each group were cultured as described above. Following this, the remaining silicone discs were re-incubated in MHB for 24 hours before receiving the same antibiotic alone or combined with rifampin for five days.

Tests to determine a person's susceptibility to certain diseases are known as susceptibility tests. In this study, MICs (minimum inhibitory concentrations) were determined for 10 MRSA isolates after exposure to all six antibiotics on a daily basis (as determined by CLSI (formerly NCCLS) recommended method) (18). Before exposure, MICs were taken prior to day 5 of growth. Daily MICs were taken thereafter until the end of growth day. It was assumed that diminished susceptibility was present if the MIC was four-fold higher than baseline after repeated exposures.

Statistical methods.

Kruskal-Wallis was used to determine whether biofilms in catheters had any inhibitory effects on MRSA (measured by the number of CFU). A significance level of less than 0.05 means that the difference is statistically significant. When Wilcoxon rank sum tests produced significant results between pairwise comparisons, we used them to discover significant differences. A sequential Bonferroni adjustment procedure was used to adjust the levels of post hoc pairwise comparisons to control for type 1 error. We used SPSS for Windows (version 12.0; SPSS, Inc., Chicago, IL) to perform the calculations.

RESULTS

After exposing MRSA in biofilms to minocycline, daptomycin, and tigecycline for 24 hours, all three antibiotics were significantly more effective at reducing MRSA than linezolid, vancomycin, and the negative control (all P values 0.001). Daptomycin and tigecycline were the next two most potent antibiotics (all P values 0.022), followed by minocycline. In a 24-hour exposure to vancomycin and linezolid, the biofilm growth of MRSA was not affected, and both drugs were indistinguishable from the broth control.
The daptomycin treatment yielded a significant reduction in biofilm colonisation by day 3 in comparison with linezolid, rifampin, and vancomycin after a short-term daily exposure of 4 hours (4 hours) per day. Fig. 1 illustrates this. The tigecycline treatment eliminated MRSA in biofilm after 3 brief 4-hour daily exposures. However, after 5 exposures, the MRSA persists in biofilm after rifampin, linezolid, and vancomycin are all considered significant.

Compared to other antibiotics, rifampin was a unique medication. The use of rifampin decreased the microbial burden of MRSA colonisation in biofilms on day 1 after an exposure lasting 4 hours. Following daily exposures of 4 hours for 5 days, rifampin was the least effective of all tested antibiotics, including linezolid and vancomycin (all P values were 0.01).

Rifampin alone on a daily basis was not able to decrease the MRSA microbial load in biofilms (Table 1.1) when used alone. A total of seven of the nine MRSA isolates that were initially highly sensitive to rifampin later developed resistance(Table (Table1.1)1). One MRSA isolate showed decreased linezolid susceptibility and three others showed reduced susceptibility. The susceptibilities to other antibiotics did not change, in particular daptomycin, minocycline, and tigecycline, which all prevented biofilm formation after three days of cyclic exposure (Fig. 1).

TABLE 1:MIC comparison of MRSA strains on day 1 and day 5

MRSA strain	MIC[a] (μg/ml) for indicated antibiotic and day

	Rifampin		Linezolid	
	Day 1	Day 5	Day 1	Day 5
4803	<0.06	128	0.5	NG
4913	128	128	0.5	8
4930	<0.06	<0.06	0.5	NG
5098	<0.06	128	0.25	4
5004	<0.06	128	0.5	NG
859	<0.06	32	0.5	2
4875	<0.06	128	0.5	NG
4342	<0.06	128	1	NG
293	<0.06	128	1	NG
789	<0.06	NG	0.5	NG

On day 1 of treatment with varied combinations of rifampin after four hours of exposure, MRSA biofilms on 10 MRSA isolates were 100 percent eradicated with rifampin combined with daptomycin, minocycline, or tigecycline. Rifampin was shown to work synergistically with vancomycin or linezolid in eradicating MRSA in biofilms following four hours of daily exposure. This suggests that rifampin is efficient at eradicating MRSA in biofilms in combination with these antibiotics.

DISCUSSION

According to this study, diptomycin, minocycline, and tigecycline inhibited MRSA germs that had hidden within a biofilm significantly better than vancomycin or linezolid. Rifampin is the least effective antibiotic for reducing biofilm colonization of MRSA after five consecutive days of daily four-hour exposure to it. The eradication of MRSA colonisation in biofilms was, however, accelerated when rifampin was combined with other antibiotics.

ALT was recommended (16) for the salvage of CVC associated with CRBSI by the Infectious Diseases Society of America (IDSA). In contrast, these recommendations took no consideration of what antibiotics are more effective for biofilm conditions or how long drugs should be locked in. It's not realistic or feasible for patients to stay in the hospital for more than 12 hours. Antibiotics for ALT are typically selected in part based on the susceptibility of microorganisms in suspension, which may or may not indicate that an antibiotic is effective against the same microorganisms embedded in biofilms.

According to previous research, vancomycin does not eradicate MRSA germs trapped in biofilm. It has been found that vancomycin has a limited effect on staphylococci in biofilms (6, 8, 9, 19, 27). In spite of several clinical trials showing promising results, antibiotic and heparin use for ALT in staphylococcal CRBSI has been associated with low salvage rates (1, 12, 15). Additionally, extended vancomycin therapy for CRBSI related to MRSA has been associated with development of vancomycin-resistant S. aureus (3). This means that vancomycin alone may not be the best treatment option for ALT that regularly results in catheter salvage.

There was also linezolid resistance in isolates of MRSA embedded in biofilms attached to silicone discs. In vitro studies had also shown this resistance. Staphylococcus epidermidis can be effectively treated with linezolid after a dwell time of over 72 hours, which is not typically feasible therapeutically (6). Linezolid and vancomycin did not affect staphylococci within biofilms, as reported by Wiederhold et al. (27). It may be less desirable to use linezolid alone as a catheter lock solution due to MRSA isolates with less susceptibility to linezolid after repeated daily exposure.

The antibiotic minocycline however, is exceptionally effective against biofilm-encrusted isolates of MRSA. As compared with vancomycin or vancomycin combined with heparin, minocycline was less effective in colonizing Staphylococcus aureus and S. epidermidis on catheter surfaces in an in vitro model (20). By adding EDTA, minocycline demonstrated even greater antistaphylococcal activity in the same in vitro model. It was successful in removing staphylococcal organisms embedded within biofilm (20) when minocycline and EDTA were combined (20). CRBSI and colonisation risk were lower with minocycline-EDTA after clinical trials (2) and (5) in patients receiving hemodialysis or paediatric cancer treatment, respectively.

Rifampin was significantly improved by adding it to minocycline in this study. All 10 isolates of bacteremic bacteria were totally prevented from colonizing biofilm with MRSA by the combination of minocycline and rifampin. In an in vitro study using a modified Robbins device (23), the enhanced efficiency against MRSA bacteria trapped in biofilm was demonstrated previously. Moreover, multiple prospective, randomised clinical trials showed that minocycline and rifampin coated catheters were much more effective in preventing CRBSI (4, 7, 11, 22) than uncoated catheters or non-antiseptic catheters. Thus, minocycline may be extremely effective to avoid staphylococcal colonisation of the CVC in ALT, whether alone or in combination with other antistaphylococcal enhancers such as EDTA and rifampin.

A derivative of minocycline, tatecycline inhibits the growth of staphylococci in biofilms and MRSA (19). Tygecycline was more efficacious than daptomycin in a systemic murine model and as effective as vancomycin (19).

Additionally, daptomycin was effective in eliminating MRSA from biofilms. Daptomycin kills germs faster than vancomycin or linezolid (10), as indicated by time-kill tests. Because calcium chelators require high calcium concentrations simultaneously, it is not suitable for use with antibiotic enhancers and calcium chelators like EDTA. It can also be used with heparin for optimal results. MRSA biofilms grow faster when heparin is used, on the other hand (25).

After 4 hours or 24 hours of exposure to rifampin, silicone discs were significantly less likely to colonize with MRSA. Despite daily rifampin exposure over a 5-day period, most MRSA isolates developed resistance to rifampin (Table (Table2)1), and colonisation of MRSA was maintained despite daily rifampin treatments (Figures 2B2B and 3A and B). Studies (9, 23,

24) have shown that rifampin reduces staphylococci colonization on catheter surfaces trapped within biofilms. Other studies have linked repeated exposure to rifampin to staphylococcal resistance (17, 26). As long as other antibiotics were administered with rifampin, rifampin resistance did not develop (17).

Using rifampin together with other antibiotics was found to be highly effective in the present study in eliminating MRSA colonisation on silicone disc surfaces. Figure 1. A rifampin combination prevented the acquisition of resistance to all antibiotics, including linezolid against MRSA buried in biofilms. Vancomycin, minocycline, ciprofloxacin, fusidic acid, and clindamycin have been reported to have increased antistaphylococcal activity against staphylococci buried in biofilm (23, 24). Saginur et al. analyzed antibiotic combination therapy for staphylococci-induced biofilms (24) and found rifampin to be the most common component. Therefore, rifampin is most effective when used in conjunction with other antistaphylococcal agents against staphylococci embedded in biofilm.

CONCLUSION

As a final point, antibiotics administered under the ALT protocol are effective against infections caused by biofilms. As compared to minocycline, daptomycin, and tigecycline, vancomycin and linezolid were significantly less effective in the treatment of MRSA trapped in biofilms. Rifampin failed to eliminate MRSA colonization in biofilm when used for 4 hours on a daily basis in a model that mimicked antibiotic catheter lock solution. Rifampin, however, can be used to enhance the effectiveness of antibiotics against staphylococcal bacteria. Combining it with other antibiotics allowed MRSA to be rapidly eliminated from biofilms.

REFERENCES

1. Bailey, E., N. Berry, and S. Cheesbrough. 2002. Antimicrobial lock therapy for catheter-related bacteraemia among patients on maintenance haemodialysis. J. Antimicrob. Chemother. 58615-617.
2. Bleyer, A. J., L. Mason, G. Russell, I. I. Raad, and R. J. Sherertz. 2005. A randomized, controlled trial of a new vascular catheter flush solution (minocycline-EDTA) in temporary hemodialysis access. Infect. Control Hosp. Epidemiol. 26520-524.
3. Chang, S., D. M. Sievert, J. C. Hageman, M. L. Boulton, F. C. Tenover, F. P. Downes, S. Shah, J. T. Rudrik, G. R. Pupp, W. J. Brown, D. Cardo, and S. K. Fridkin for the Vancomycin-Resistant Staphylococcus aureus Investigative Team. 2003. Infection with vancomycin-resistant Staphylococcus aureus containing the vanA resistance gene. N. Engl. J. Med. 3481342-1347.
4. Chatzinikolaou, I., K. Finkel, H. Hanna, M. Boktour, J. Foringer, T. Ho, and I. Raad. 2003. Antibiotic-coated hemodialysis catheters for the prevention of vascular catheter-related infections: a prospective, randomized study. Am. J. Med. 115352-357.
5. Chatzinikolaou, I., T. F. Zipf, H. Hanna, J. Umphrey, W. M. Roberts, R. Sherertz, R. Hachem, and I. Raad. 2003. Minocycline-ethylenediamine-tetraacetate lock solution for the prevention of implantable port infections in children with cancer. Clin. Infect. Dis. 36116-119.
6. Curtin, J., M. Cormican, G. Fleming, J. Keelehan, and E. Colleran. 2003. Linezolid compared with eperezolid, vancomycin, and gentamicin in an in vitro model of

antimicrobial lock therapy for Staphylococcus epidermidis central venous catheter-related biofilm infections. Antimicrob. Agents Chemother. 473145-3148.

7. Darouiche, R. O., I. I. Raad, S. O. Heard, J. I. Thornby, O. C. Wenker, A. Gabrielli, A. J. Berg, N. Khardori, H. Hanna, R. Hachem, R. L. Harris, and G. Mayhall for the Catheter Study Group. 1999. A comparison of two antimicrobial-impregnated central venous catheters. N. Engl. J. Med. 3401-8.
8. Evans, R. C., and C. J. Holmes. 1987. Effect of vancomycin hydrochloride on Staphylococcus epidermidis biofilm associated with silicone elastomer. Antimicrob. Agents Chemother. 32889-894.
9. Farber, B. F., M. H. Kaplan, and A. G. Clogston. 1990. Staphylococcus epidermidis biofilm associated with silicone elastomer. Antimicrob. Agents Chemother. 16137-40.
10. Fuchs, P. C., A. L. Barry, and S. D. Brown. 2002. In vitro bactericidal activity of daptomycin against staphylococci. J. Antimcrob. Chemother. 49467-470.
11. Hanna, H., R. Benjamin, I. Chatzinikolaou, B. Alakech, D. Richardson, P. Mansfield, M. Munsell, R. Darouiche, H. Kantarjian, and I. Raad. 2004. Long-term silicone central venous catheters impregnated with minocycline and rifampin decrease rates of catheter-related bloodstream infection in cancer patients: a prospective randomized clinical trial. J. Clin. Oncol. 223163-3171.
12. Krishnasami, Z., D. Carlton, L. Bimbo, M. E. Taylor, D. F. Balkovetz, J. Baker, and M. Allon. 2002. Management of hemodialysis catheter-related bacteremia with an adjunctive antibiotic lock solution. Kidney Int. 611136-1142.
13. Kuhn, D. M., T. George, J. Chandra, P. K. Mukherjee, and M. A. Ghannoum. 2002. Antifungal susceptibility of Candida biofilms: unique efficacy of amphotericin B lipid formulations and echinocandins. Antimicrob. Agents Chemother. 461773-1780.
14. Labthavikul, P., P. J. Petersen, and P. A. Bradford. 2003. In vitro activity of tigecycline against Staphylococcus epidermidis growing in an adherent cell biofilm model. Antimicrob. Agents Chemother. 473967-3969.
15. Longuet, P., M. C. Doudard, G. Arlet, J. M. Molina, C. Benoit, and C. Leport. Venous access port-related bacteremia in patients with acquired immunodeficiency syndrome or cancer: the reservoir as a diagnostic and therapeutic tool. Clin. Infect. Dis. 321776-1783.
16. Mermel, L. A., B. M. Farr, R. J. Sherertz, I. I. Raad, N. O'Grady, J. S. Harris, and D. E. Craven. 2001. Guidelines for the management of intravascular catheter-related infections. Clin. Infect. Dis. 321249-1272.
17. Munson, E. L., S. O. Heard, and G. V. Doern. 2004. In vitro exposure of bacteria to antimicrobial impregnated-central venous catheters does not directly lead to the emergence of antimicrobial resistance. Chest 1261628-1635.
18. National Committee for Clinical Laboratory Standards. 2000. Performance standards for antimicrobial susceptibility testing, 10th informational supplement. M-100-S-10. NCCLS, Wayne, PA.
19. Petersen, P. J., P. A. Bradford, W. J. Weiss, T. M. Murphy, P. E. Sum, and S. J. Projan. 2002. In vitro and in vivo activities of tigecycline (GAR-936), daptomycin, and comparative antimicrobial agents against glycopeptide-intermediate Staphylococcus aureus and other resistant gram-positive pathogens. Antimicrob. Agents Chemother. 462595-2601.
20. Raad, I., I. Chatzinikolaou, G. Chaiban, H. Hanna, R. Hachem, T. Dvorak, G. Cook, and W. Costerton. 2003. In vitro and ex vivo activities of minocycline and EDTA against microorganisms embedded in biofilm on catheter surfaces. Antimicrob. Agents Chemother. 473580-3585.

21. Raad, I., W. Costerton, U. Sabharwal, M. Sacilowski, E. Anaissie, and G. P. Bodey. 1993. Ultrastructural analysis of indwelling vascular catheters: a quantitative relationship between luminal colonization and duration of placement. J. Infect. Dis. 168400-407.
22. Raad, I., R. Darouiche, J. Dupuis, A. Gabrielli, D. Abi-Said, R. Hachem, M. Wall, R. Harris, J. Jones, A. Buzaid, C. Robertson, S. Shenaq, P. Curling, T. Burke, C. Ericsson, and the Texas Medical Center Catheter Study Group. 1997. Central venous catheters coated with minocycline and rifampin for the prevention of catheter-related colonization and bloodstream infections. Ann. Intern. Med. 127267-274.
23. Raad, I., R. Darouiche, R. Hachem, M. Sacilowski, and G. P. Bodey. 1995. Antibiotics and prevention of microbial colonization of catheters. Antimicrob. Agents Chemother. 392397-2400.
24. Saginur, R., M. St. Denis, W. Ferris, S. D. Aaron, F. Chan, C. Lee, and K. Ramotar. 2006. Multiple combination bactericidal testing of staphylococcal biofilms from implant-associated infections. Antimicrob. Agents Chemother. 5055-61.
25. Shanks, R. M. Q., N. P. Donegan, M. L. Graber, S. E. Buckingham, M. E. Zegans, A. L. Cheung, and G. A. O'Toole. 2005. Heparin stimulates Staphylococcus aureus biofilm formation. Infect. Immun. 734596-4606.
26. Tambe, S. M., L. Sampath, and S. M. Modak. 2001. In vitro evaluation of the risk of developing bacterial resistance to antiseptics and antibiotics used in medical devices. J. Antimicrob. Chemother. 47589-598.
27. Wiederhold, N. P., E. A. Coyle, I. I. Raad, R. A. Prince, and R. E. Lewis. 2005. Antibacterial activity of linezolid and vancomycin in an in vitro pharmacodynamic model of gram-positive catheter-related bacteraemia. J. Antimicrob. Chemother. 55792-795.