Combination of Tigecycline and N-acetylcysteine Reduces Biofilm-Embedded Bacteria on Vascular Catheters

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ABSTRACT

To assess the efficacy of anti-biofilm/antimicrobial combination, we incubated catheter segments colonized with one of six studied bacterial organisms in N-acetylcysteine, tigecycline, N-acetylcysteine/tigecycline combination, or saline. Segments were washed, sonicated and cultured. N-acetylcysteine/tigecycline combination significantly decreased all viable biofilm-associated bacteria and was synergistic for methicillin-resistant *Staphylococcus aureus* and *S. epidermidis*.

Word Count: 50 words
Vascular catheter-associated bacteremia has a substantial impact on morbidity, mortality, duration of stay and overall cost of health care. (4, 16, 23) Infections due to biofilm-embedded bacteria are difficult to eradicate without removal of the infected device. (10, 12) However, other access sites may not be available and patients may not wish to undergo further procedures. In instances where removal of an infected tunneled vascular catheter is not feasible or desirable, a trial of an antibiotic lock solution (plus systemic antibiotics) can be considered for treatment of uncomplicated bacteremia. (12) Since this practice has not been uniformly successful, (7, 17, 19) there is a need to assess other strategies, such as the use of anti-biofilm/antimicrobial catheter lock solution to salvage infected vascular catheters.

N-acetylcysteine (NAC) decreases biofilm formation due to a variety of bacteria (9, 15, 20) and reduces extracellular polysaccharide matrix production (14) while promoting disruption of mature biofilm. (9, 20) NAC is widely used in medical practice via inhalation, oral and intravenous routes (11, 13, 27) and has an excellent safety profile. (6) Tigecycline is active against a range of multi-resistant organisms and is bactericidal against biofilm-associated Staphylococcus epidermidis at a lower minimal bactericidal concentration than both vancomycin and daptomycin. (8)

We hypothesized that the combination of NAC and tigecycline is synergistic in the treatment of catheter-associated biofilm, as they both act on different components of the biofilm.

We used 6 microorganisms that had been isolated from patients with catheter-associated bacteremia. These were methicillin-resistant S. aureus (MRSA), methicillin-sensitive S. aureus...
(MSSA), methicillin-resistant *S. epidermidis* (MRSE), vancomycin-resistant enterococcus (VRE), *Klebsiella pneumoniae*, and *Acinetobacter baumannii*. We purchased 20% N-acetylcysteine (Hospira, Inc., Lake Forest, IL) and lyophilized tigecycline (Wyeth Pharmaceuticals Inc., Philadelphia, PA). We used 80 mg/ml of NAC based on preliminary in-vitro data which showed a dose-response relationship on planktonic bacteria (unpublished) and 1 mg/ml of tigecycline (this dose is a 1000-fold higher than its minimum inhibitory concentration (MIC) for the organisms tested in the planktonic phase).

**Four-centimeter** segments of 7-French, triple-lumen central venous catheters (Cook Inc., Bloomington, IN) were incubated in bacterial suspensions that contained $10^5$ CFU/ml of bacteria in trypticase soy broth (TSB) to allow biofilm formation. After incubation at 37°C for 24 hours, segments were removed and excess broth was shaken off. One catheter segment was rinsed and cultured to obtain a baseline value and the remaining segments were suspended for 4 or 12 hours at 37°C in one of the following treatment solutions: N-acetylcysteine, tigecycline, combination of NAC and tigecycline, and normal saline (NS) as control.

**Catheter** segments were rinsed thrice with NS to remove planktonic bacteria. The distal and proximal 0.5-cm ends were cut and the remaining segment was divided into three 1-cm sections. These sections were individually sonicated for 10 minutes and vortexed for 30 seconds in 1 ml of phosphate buffered saline (PBS). 100 µl aliquots of the original sonication fluid and successive dilutions were inoculated onto blood agar plates (limit of detection - 10 CFU). Bacterial colonies were counted after incubation for 24 hours. The median colony count of the three 1-cm sections from the same 4-cm segment was considered as the representative value for that segment. Each
A set of experiments was repeated five times. The CFU/cm of catheter for different groups was compared by the Mann Whitney U test (Stata version 9, StataCorp, College Station, TX) and p < 0.05 indicated significance.

We recovered $10^4$-$10^5$ CFU/cm of bacteria from catheter-associated biofilm at 24 hours for all organisms except for VRE, which consistently had $10^3$ CFU/cm (figure 1). NAC had an independent antimicrobial effect on biofilm-associated MRSA, MRSE and *K. pneumoniae* (p < 0.01 compared to control at 12 hours). For other organisms, the effect of NAC alone was similar to control. Tigecycline alone significantly reduced the CFU of all tested bacteria when compared to control (p < 0.05), except for VRE.

**NAC/tigecycline** combination consistently decreased viable biofilm-associated bacteria when compared to control. This combination was synergistic for MRSA at 4 hours (p < 0.01) and MRSE at 12 hours (p < 0.05). We were unable to detect any bacteria in cases of MRSA, MRSE, MSSA and *K. pneumoniae* after incubation with the combination.

**Utilizing** high doses of antimicrobials to eradicate biofilm has had limited success in the clinical setting. (7, 19) This may be due to inadequate penetration of antibiotics, higher MIC of antibiotics for biofilm-associated bacteria, reduced growth rate, and local alterations in the biofilm environment that impair activity of the antibiotic. (2, 3, 24) Since antimicrobial susceptibility of biofilm-associated *S. epidermidis*, MRSA and MSSA is enhanced in disrupted biofilm, (5) it is conceivable that an anti-biofilm/antimicrobial combination would be synergistic. By degrading the extracellular polysaccharide matrix of biofilm, (9, 14) it is possible that NAC
may have made the biofilm-associated bacteria more susceptible to tigecycline, although we did not specifically test this hypothesis. Our NAC/tigecycline combination was synergistic for *S. aureus* and *S. epidermidis* - the two organisms that are most commonly associated with vascular catheter-related infections. (4, 17)

A similar 4-5 log_{10} decrease in viable cells dislodged from catheters has been described after incubation for 24 hours with other lock solutions such as minocycline/rifampin, ciprofloxacin/rifampin, (22) taurolidine/citrate, (21) and in some cases, minocycline/EDTA. (18) The NAC/tigecycline combination compared favorably to vancomycin and linezolid that led to only a 1 log_{10} and 3 log_{10} decrease, respectively, in biofilm-embedded *S. epidermidis* after incubation for 24 hours, (1) 2 log_{10} decrease in *S. aureus* after 24 hour incubation with each drug, (26) and 2 log_{10} decrease in VRE after incubation with linezolid for 24 hours. (26) Although most previous studies evaluating catheter lock solutions have used a continuous dwell time anywhere from 24 to 336 hours to achieve sterilization of the biofilm, (1, 18, 25) we used a shorter dwell time of 12 hours which is more clinically feasible.

The promising in-vitro results prompted us to initiate a single-arm pilot clinical trial evaluating the use of NAC/tigecycline combination as a catheter lock solution for the treatment of catheter-associated bacteremia arising from tunneled hemodialysis catheters. Should the clinical trial demonstrate efficacy and safety of this innovative catheter lock solution, we plan to explore the use of NAC/tigecycline in a randomized fashion.

**ACKNOWLEDGEMENT**
The authors have no financial conflicts of interest.
REFERENCES


tigecycline against Staphylococcus epidermidis growing in an adherent-cell biofilm model.

Schito. 2003. Effect of fosfomycin alone and in combination with N-acetylcysteine on E. coli

Kirkland. 1997. Catheter-related bacteremia and outcome of attempted catheter salvage in


E. Hilleman. 2003. Acetylcysteine in the prevention of contrast-induced nephropathy after

growth, extracellular polysaccharide production, and bacterial biofilm formation on solid

A. C. Gomez-Garcia. 1997. Influence of N-acetylcysteine on the formation of biofilm by


Legend for Figure 1. The effect of normal saline control, N-acetylcysteine (NAC), tigecycline, and the combination of NAC and tigecycline on bacterial colony counts of different organisms. The bars represent mean CFU/cm of catheter at different time points along with respective standard error of the mean. The y-axis is on a logarithmic scale. The horizontal line denotes the lower limit of detection (LLD) of 10 CFU/cm. * p < 0.05 when compared to the control at the same time. § p < 0.05 when compared to the combination at the same time. (Excel was used to create the graph)
Figure 1

**MRSA**

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**Klebsiella pneumoniae**

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