In Vitro Activity and Durability of a Combination of an Antibiofilm and an Antibiotic against Vascular Catheter Colonization

Mohammad D. Mansouri,a,b Richard A. Hull,a Charles E. Stager,a,b Richard M. Cadle,a,b Rabih O. Darouichea,b
Baylor College of Medicine, Houston, Texas, USAa; Michael E. DeBakey Veterans Affairs Medical Center, Houston, Texas, USAa

Catheter-associated infections can cause severe complications and even death. Effective antimicrobial modification of catheters that can prevent device colonization has the potential of preventing clinical infection. We studied in vitro the antimicrobial activities of central venous catheters impregnated with N-acetylcysteine (NAC), an antibiofilm agent, and a broad-spectrum antibiotic against a range of important clinical pathogens. NAC-levofloxacin-impregnated (NACLEV) catheters were also evaluated for their antiadherence activity. NACLEV catheters produced the most active and durable antimicrobial effect against both Gram-positive and Gram-negative isolates and significantly reduced colonization (P < 0.0001) by all tested pathogens compared to control catheters. These in vitro results suggest that this antimicrobial combination can potentially be used to combat catheter colonization and catheter-associated infection.

Intravascular catheters are essential in managing critically ill patients. However, vascular catheter-associated infections could result in dire consequences leading to excessive morbidity and mortality, longer hospital stays, and higher health care costs, with an average treatment cost of $25,000 per episode (1–5).

Catheter-associated infections are generally initiated by microbial colonization of the catheter surface and formation of a superficial biofilm layer (6). Cell surface proteins and polysaccharide production by bacterial cells reportedly contribute to the formation of biofilm that can then protect pathogens by impeding both the penetration of antibiotics and the function of phagocytic immune cells, thus hindering the ability to combat colonizing pathogens (7–9).

Impregnation or coating of catheters with antimicrobial agents has commonly been used to prevent bacterial colonization of vascular catheters (8). However, some existing antimicrobial-treated catheters designed to prevent catheter colonization may have partial clinical efficacy, particularly against drug-resistant pathogens, and limited durability of antimicrobial activity (8, 10–13) partly due to their inability to control biofilm formation and combat biofilm-nested microorganisms, which can have MICs of up to 1,000 times higher than their MICs against their free-floating planktonic counterparts (8, 14–16).

N-acetylcysteine (NAC), a commonly used inhalation mucolytic therapy for chronic bronchitis and an FDA-approved intravenous injection for the treatment of acetaminophen toxicity (17), has favorable pharmacokinetics when used in hemodialysis patients (18). NAC also adversely affects bacterial growth and polysaccharide production and disrupts disulfide bonds in mucus, reducing the viscosity of secretions. These properties may contribute to the prevention and disruption of biofilm around different polymeric and metallic surfaces (9, 19–21). Not only does NAC diminish the formation of biofilm by common pathogens (19, 22, 23), it also possesses some in vitro intrinsic antimicrobial activity against both Gram-positive and Gram-negative bacteria (24). Taking into consideration the therapeutic safety record and the antibiofilm ability of NAC combined with antimicrobial activity of a broad-spectrum antibiotic, impregnation of intravascular catheters with this unique combination can be a promising approach for reducing catheter colonization and potential subsequent catheter-associated infection.

Seven-French, triple-lumen, polyurethane central venous catheter (CVC) segments (Cook Inc., Bloomington, IN) were impregnated with a combination of NAC and levofloxacin, neomycin, or gentamicin by immersion in an agitated solution that comprised 100 mg/ml of NAC and 100 mg/ml of the antibiotic, followed by drying overnight and rinsing to remove any unbound compound (25). Since the size of the zone of inhibition (ZI) generated by antimicrobial-coated devices is reportedly correlated with their in vivo antimicrobial efficacy to prevent colonization (26), we assessed the antimicrobial activity of impregnated catheter segments at baseline and after exposure to human serum over different periods of time against a panel of important clinical isolates. One-centimeter segments of NAC-antibiotic-impregnated and nonimpregnated control catheters were individually placed in human serum at 37°C for 3, 7, 10, 14, and 30 days, with a weekly change of serum. Catheter segments removed from sera and baseline catheter segments (no serum incubation) were assessed for ZI, using a modified Kirby-Bauer diffusion assay (27–29), against methicillin-resistant Staphylococcus epidermidis (MRSE), methicillin-resistant S. aureus (MRSA), vancomycin-resistant Enterococcus (VRE), Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli. Briefly, bacterial suspensions at 10⁶ CFU/ml were streaked uniformly onto Mueller-Hinton agars using cotton swabs, and 1-cm catheter segments were then half embedded in the center of agar plates, which were then incubated at 37°C overnight. These in vitro experiments were designed to select for further evaluation the most active and durable NAC-antibiotic combination with the broadest spectrum of antimicrobial activity.

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segments displayed the most potent (ZI > 15 mm), widest spectrum, and longest durability of antimicrobial activity against tested pathogens, we further evaluated their antiadherence efficacy by culture methods and microscopic observations.

We assessed the anticolonization activity of NACLEV catheters by using a modified Robbins device (MRD) (24) that consists of an acrylic apparatus with an enclosed internal groove that can sequentially accommodate up to 25 1-cm catheter segments. Variations of this device have been described in other studies (30–32). Twenty 1-cm segments of NACLEV or control catheters were steriley placed in the MRD in sequence. The MRD was then closed, sealed, and connected to a circulating stream of bacterial suspension of MRSE, MRSA (levofloxacin sensitive), MRSA (levofloxacin resistant), VRE, *P. aeruginosa*, *K. pneumoniae*, or *E. coli* at 2 × 10^5 CFU/ml using a peristaltic pump with a flow rate of 1 ml/min around and through the lumens of each catheter segment for 24 h at 37°C. The system was then flushed with one MRD volume of normal saline. Segments were then removed, individually placed in 1 ml of normal saline, and cultured quantitatively using the standard sonication technique (24, 28). Since the MRD device could only accommodate one bacterial suspension and either the impregnated or nonimpregnated catheter segments, this process was repeated for each organism and for each type of catheter. We specifically tested the activity of NACLEV catheters against levofloxacin-resistant MRSA to evaluate the possible synergetic antimicrobial effect of this combination.

To visualize the antibiotic film and antibacterial effects of the NAC-levofloxacin combination, two 0.5-cm segments of NACLEV or control catheters were individually incubated in a 2 × 10^5-CFU/ml suspension of a Gram-positive (MRSA) or Gram-negative (*K. pneumoniae*) representative pathogen at 37°C overnight. Catheter segments were then briefly rinsed with normal saline, fixed in a glutaraldehyde buffer solution, dehydrated, and observed under a JEOL (Peabody, MA) NeoScope JCM-5000 scanning electron microscope (SEM) (33). Samples were coded, and the SEM operator was blinded in regard to the catheter impregnation and treatment. The degree of catheter colonization was assessed comparatively between the impregnated and control segments based on the clusters of organisms and the amount of superficial biofilm observed.

We used Stata software (Stata Corp., College Station, TX) for our statistical analyses. *P* values of ≤0.05 indicated a statistical significance.

In general, NACLEV catheters displayed larger zones of inhibition against all tested pathogens compared to NAC-neomycin- or NAC-gentamicin-impregnated catheters, both at baseline and after incubation in human serum for different time periods. Representative images of these zones are shown in Fig. 1a to c. NACLEV catheter segments generally produced the largest ZIs against MRSA, a very important pathogen associated with catheter-related infections, compared to NAC-gentamicin- or NAC-neomycin-impregnated catheters at each time period. NAC-gentamicin-impregnated catheters had larger ZIs than NAC/ neomycin-impregnated catheters at baseline and at different time periods after exposure to human serum. Nonimpregnated control catheter segments did not produce any zone of inhibition against any organism. The graphical antimicrobial durability of these catheters is depicted in Fig. 2a to f.

Since NACLEV catheters displayed the most potent and durable antimicrobial activity against all test pathogens, we selected this combination for further evaluation and showed that this combination significantly (*P* < 0.0001) reduced all bacterial colonization compared to control catheter segments. NACLEV catheter segments exposed to *P. aeruginosa* were completely sterile, whereas the control segments averaged 1.3 × 10^6 CFU/segment. More importantly, NACLEV catheters significantly (*P* < 0.0001) reduced catheter colonization by levofloxacin-resistant MRSA. The mean colony counts extracted from catheter segments are displayed in Fig. 3.

SEM images revealed that considerably fewer bacterial cells of MRSA and *K. pneumoniae* were attached to NACLEV catheters than control catheter segments (Fig. 4a to d). In addition, there were less visible biofilms observed on NACLEV catheter segments versus nonimpregnated control segments when exposed to either organism.

Despite many infection control measures and implementation of guidelines, the prevention of infections associated with medical devices, particularly vascular catheters, remains challenging. The undue morbidity, excessive mortality, and rising cost associated with managing catheter-associated bloodstream infections are staggering (34, 35). Patients often spend 10 to 40 additional days in hospitals as a result of acquiring these infections (36–38). However, recently established U.S. Medicare policy changes prohibit reimbursing hospitals and health care facilities for the cost of managing certain health care-acquired complications that are reasonably preventable, including vascular catheter-associated bloodstream infections (39). Furthermore, the Guidelines for the Prevention of Intravascular Catheter-Related Infections issued by the Centers for Disease Control and Prevention (CDC) and the Infectious Diseases Society of America (IDSA) recommend the

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**FIG 1** (a to c) Zones of inhibition produced by NAC-antibiotic-impregnated catheter segments after incubation in serum.
The use of antimicrobial-impregnated CVCs in patients whose catheters are expected to remain indwelling for more than 5 days if, after successful implementation of a comprehensive strategy to decrease rates of central line-associated bloodstream infection, the rate remains above the goal set by the individual institution (5). A meta-analysis showed that currently available anti-infective CVCs still have an overall colonization rate of about 14% (345/2,491) compared to a rate of 24% (607/2,524) for standard untreated catheters (13). Furthermore, the duration of antimicrobial activity sharply decreases with a catheter’s indwelling time. The overall colonization rates of indwelling anti-infective versus standard catheters were 12% versus 24%, 23% versus 28%, and 17% versus 16% for catheters placed for 5 to 12 days, 13 to 20 days, and >20 days, respectively. After 20 days of catheter placement, there is virtually no difference in the colonization rates between antimicrobial-treated and untreated standard catheters (13).

Since pathogenic colonization of a catheter can occur days after its implantation, it would be optimal for antimicrobial-treated catheters to remain indwelling for no more than 5 days.
catheters to have a sustained activity for as long as it is safe and necessary. However, the activities of many antimicrobial-treated catheters usually diminish rapidly during the first few days of placement in patients due to the rapid and uncontrolled release of the antimicrobial agents into the bloodstream. A measured release can ensure a sustained antimicrobial activity against microorganisms as reflected by the durable zones of inhibition produced by NACLEV catheters after incubation in human serum in vitro. SEM colonization images corroborate the adherence assay findings, indicating that NACLEV catheters substantially reduce bacterial colonization and biofilm formation by both Gram-positive and Gram-negative bacteria.

NACLEV catheter segments exposed to bacterial suspensions displayed 53-fold (with VRE) to more than $1.2 \times 10^6$-fold (with *E. coli*) fewer average numbers of adherent bacterial cells than control catheter segments and completely impeded adherence of *P. aeruginosa* bacterial cells. Although levofloxacin alone is clinically effective against a small percentage of clinical cases of MRSA infections, the incorporation of sufficient and safe concentrations of levofloxacin and NAC into catheters may synergistically alleviate this limitation based on the strong in vitro anticolonization activity of NACLEV catheters against levofloxacin-resistant MRSA in the MRD. Since our MRD could accommodate all segments from each comparing group (impregnated or nonimpregnated) at each run, we eliminated the need to perform multiple independent observations, promoting consistency in our results.

These promising in vitro results suggest that NACLEV catheters may protect against catheter colonization, without which catheter-associated infection would not evolve. Although statistically significant, these data may not translate into clinical significance, and therefore, it is important to evaluate these catheters in vivo as well. In light of these favorable results, we have initiated an animal study to evaluate the efficacy of NACLEV catheters against catheter colonization and catheter-associated infection. Future research will also assess the safety of this novel approach.

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Baylor College of Medicine (Houston, TX) is the assignee for patents that describe the method of incorporating agents onto catheters and the use of the combination of NAC and antibiotics. M.D.M. and R.O.D., employees of the mentioned institution, are inventors of one or more of these patents. However, there is no licensing activity regarding these patents at this time.
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