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

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Running Title: An antibiofilm/antibiotic against catheter colonization

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ABSTRACT

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 as 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 (6, 8, 14, 17, 24).

Catheter-associated infections are generally initiated by microbial colonization of the catheter surface and formation a superficial biofilm layer (16). 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 (5, 7, 26).

Impregnation or coating of catheters with antimicrobial agents has commonly been used to prevent bacterial colonization of vascular catheters (7). 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 (7, 10-13) partly due to their inability to control biofilm formation and combat biofilm-nested microorganisms, which can have minimum inhibitory concentrations (MIC) of up to 1000 times higher than their MIC against their free-floating planktonic counterparts (1, 7, 35, 39).

N-acetylcysteine (NAC), a commonly used inhalation mucolytic therapy for chronic bronchitis and an FDA-approved IV injection for the treatment of acetaminophen toxicity
(36), has favorable pharmacokinetics when used in hemodialysis patients (37). 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 (22, 25-26, 38). Not only NAC diminishes the formation of biofilm by common pathogens (22, 27, 34), it also possesses some in vitro intrinsic antimicrobial activity against both Gram-positive and Gram-negative bacteria (20). 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 CVC catheter segments (Cook Inc., Bloomington, IN) were impregnated with a combination of NAC and levofloxacin, neomycin, or gentamicin by immersion in an agitated solution that comprises 100 mg/ml of NAC and 100 mg/ml of the antibiotic followed by drying overnight and rinsing to remove any unbound compound (21). 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 (2), 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-cm segments of NAC/antibiotic-impregnated and non-impregnated control catheters were individually placed in human serum at 37°C for 3, 7, 10, 14, and 30 days, with 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 (18-19, 32), 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 $10^8$ cfu/ml were streaked uniformly onto Mueller-Hinton agars using cotton swabs, and one-cm segments were then half embedded in the center of agar plates, which were then incubated at 37ºC 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.

Since NAC/levofloxacin-impregnated (NACLEV) catheter segments displayed the most potent (ZI ≥15 mm), widest spectrum, and longest durability of antimicrobial activity against tested pathogens, we further evaluated their anti-adherence efficacy by culture methods and microscopic observations.

We assessed the anti-colonization activity of NACLEV catheters by using a modified Robbins device (MRD) (20) that consists of an acrylic apparatus with an enclosed internal groove that can sequentially accommodate up to twenty-five 1-cm catheter segments. Variations of this device have been described in other studies (15, 23, 33). 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 X 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 hours 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 (19-20). Since the MRD device could only accommodate one bacterial suspension and either the impregnated or non-impregnated 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 possible synergetic antimicrobial effect of this combination.

To visualize the antibiofilm and antibacterial effects of the NAC-levofloxacin combination, two 0.5-cm segments of NACLEV or control catheters were individually incubated in 2 X10^5 cfu/ml suspension of a Gram-positive (MRSA) or a Gram-negative (*K. pneumoniae*) representative pathogen at 37°C overnight. Catheter segments were then briefly rinsed with normal saline, fixed in a gluteraldehyde buffer solution, dehydrated, and observed under a JEOL (Peabody, MA) NeoScope JCM5000 scanning electron microscope (SEM) (4). Samples were coded and the SEM operator was blinded in regards 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 <0.05 indicated statistical significant.

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-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 compared to NAC/neomycin-impregnated catheters at baseline and at different time periods after exposure to human serum. Fig. 2a-f depicts graphical antimicrobial durability of these catheters.

Since NACLEV catheters displayed the most potent and durable antimicrobial activity against all test pathogens, we selected this combination for further evaluations 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 X 10⁶ 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 compared to control catheter segments (Fig. 4a-d). In addition, there were less visible biofilms observed on NACLEV catheter segments vs. non-impregnated control segments when exposed to either organism.

Despite many infection control measures and implementation of guidelines, 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 (29-30). Patients often spend 10-40 additional days in hospitals as a result of acquiring these infections (9, 28, 31). However, recently established US Medicare policy changes prohibit reimbursing hospitals and healthcare facilities for the cost of managing certain healthcare-acquired complications that are reasonably preventable, including vascular catheter-associated bloodstream infections (3). 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 use of antimicrobial-impregnated CVC in patients whose catheter is expected to remain indwelling for more than five 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 (24). A meta-analysis showed that currently available anti-infective CVC still have an overall colonization rate of about 14% (345/2491) compared to a rate of 24% (607/2524) 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 vs. standard catheters were 12% vs. 24%, 23% vs. 28%, and 17% vs. 16% for catheters placed for 5-12 days, 13-20 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 have a sustained activity for as long as 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 X 10^6 fold (with E. coli) fewer average number of adherent bacterial cells compared to 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, 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 non-impregnated) 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, Texas, USA) 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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FIG 1a–c Zones of inhibition produced by NAC/antibiotic-impregnated catheter segments after incubation in serum.
FIG 2a-f Antibacterial activity of NAC/antibiotic-impregnated central venous catheters against common clinical pathogens based on zone of inhibition.
FIG 3. Anticolonization activity of NAC/levofloxacin-impregnated catheter segments exposed to different bacterial suspensions in MRD. Error bars indicate standard error of means (SEM) for bacterial colony counts per cm of catheter.

*Significant difference in mean bacterial colony counts (P < 0.0001) between control and NACLEV devices.
FIG 4a-b Catheter segment incubated in MRSA

FIG 4c-d Catheter segment incubated in Klebsiella pneumoniae