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N-acetylcysteine Lock Solution Prevents Catheter-Associated Bacteremia in Rabbits

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Abstract

Purpose—Indwelling vascular catheters are the most common cause of nosocomial bloodstream infections. One approach to infection prevention is the use of antimicrobial catheter lock solutions though their widespread use is limited secondary to concern for emergence of antimicrobial resistance. The primary purpose of this study was to determine the efficacy of N-acetylcysteine (NAC) lock solution in preventing peripheral bacteremia using an in vivo model of catheter-associated infection.

Methods—Twenty-four hours after inoculating a clinical isolate of *Staphylococcus aureus* into the lumen of tunneled external jugular catheters in rabbits, a catheter lock solution that contained NAC vs. heparinized saline alone was allowed to dwell for two consecutive periods of 72 hours. Surveillance peripheral and centrally collected blood cultures were obtained. Distal intravascular segments of the catheters were removed at day 7 and cultured using a sonication method.

Results—At 7 days after catheter insertion, none of the NAC-treated rabbits (0/8) developed peripheral bacteremia with *S. aureus* whereas 4/7 controls did ($p=0.02$). The bacterial yield from catheter tip cultures was not statistically different in both arms.

Conclusions—These promising data encourage further clinical evaluation of NAC lock solution for prevention of catheter-associated bacteremia in patients.

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Conflict of Interest: S.A., K.J., S.R., R.M. and M.G. do not have any conflicts of interest. R.O.D. has assigned the rights of a patent describing the use of N-acetylcysteine to combat device-related infections to his employer, Baylor College of Medicine. There is currently no licensing agreement regarding this patent between Baylor College of Medicine and any company, hence, there is no receipt of royalties.

Keywords

N-acetylcysteine; lock solution; catheter-associated bacteremia

Introduction

Indwelling vascular catheters are the most common cause of nosocomial bloodstream infections, particularly in hemodialysis-dependent subjects. Review of national surveillance data from the United States reveals that 20% of patients with end-stage renal disease (ESRD) used central venous catheters (CVCs) for hemodialysis in 2007.(1) Use of CVCs for hemodialysis is associated with 1.5 catheter infections per patient year and is a predictor of all-cause and infection-specific mortality in these patients.(1, 2) Since the population at risk for hemodialysis catheter-associated bacteremia (CAB) has greatly increased in recent years, infection prevention is crucial.

Use of various antimicrobial catheter lock solutions is effective at reducing the incidence of CAB.(3–6) However, concern for introduction of antimicrobial resistance has prevented widespread adoption of this technique. Thus, there is an ongoing interest in exploring non-antibiotic alternatives as one of the methods of infection prevention.

We previously demonstrated the efficacy of N-acetylcysteine (NAC) against planktonic and biofilm-embedded organisms in vitro.(7, 8) In a single-arm pilot trial, we also successfully treated patients with hemodialysis CAB by combining systemic antibiotic therapy with a lock solution that comprised of NAC, tigecycline, and heparin.(9) The goal of the current experiment was to determine the efficacy of NAC as a catheter lock solution in preventing CAB in an animal model before conducting human studies.

Materials and Methods

All animal experiments were performed according to guidelines from the Institutional Animal Care and Use Committee at the University of California, San Diego, California, USA.

Procedure

As depicted in the study time-line (Figure 1), fifteen New Zealand White, specific pathogen-free, female rabbits (body weight 3–5 kg) underwent placement of tunneled CVCs and then received an intraluminal inoculum of 300 µl of 10³ CFU/ml of a biofilm-forming strain of *Staphylococcus aureus* suspended in heparinized saline (HS). After 24 hours, the bacterial suspension was aspirated and discarded. Rabbits were divided into two groups: interventional group (n=8) received 500 µl of NAC lock solution and the control group (n=7) received 500 µl of HS (1000 units/ml) alone. Lock solution was placed on days 1 and 4 for 72 hours each (the time period was chosen to simulate hemodialysis patients). Peripheral and central blood cultures were obtained on days 1, 4 and 7 following removal of the inoculum or lock solution. Rabbits were monitored daily for signs and symptoms of infection. They were humanely euthanized on day 7, and catheters were aseptically removed for culture via sonication.

Catheters

Silastic tubing, 0.04 inch internal diameter, was cut to 20 cm sections and a 1-cm polyethylene cuff was slipped over the catheter 4 cm from the distal end and superglued in position. The catheters were gas sterilized before insertion.

Catheter lock solution

The investigational lock solution consisted of NAC 80 mg/ml (Acetadote, Cumberland Pharmaceuticals Inc., Nashville, Tennessee, USA) and HS 1000 units/ml. The control solution contained HS 1000 units/ml. The solutions were aseptically prepared.

Operative Procedure

Anesthesia was induced with an intramuscular injection of ketamine 35 mg/kg and xylazine 5mg/kg. Rabbits were intubated and received isoflurane gas (1.5–3%) for the duration of surgery. After applying ocular lubricant, the right cervical and scapular areas were shaved with clippers, and the area washed with betadine soap, isopropyl alcohol, and betadine solution. Surgery was conducted under sterile conditions. A segment of the external jugular vein distal to the bifurcation of the internal and external maxillary veins was dissected free. Two sections of 3–0 vicryl suture were looped around the isolated vein segment. The catheter was inserted through an incision in the vein segment via a plastic introducer to the level of the cuff. Proximal and distal (over the cuff) ligatures were tied. The free edge of the catheter was passed through a subcutaneous tunnel to the intrascapular region. A luer adapter and luer lock injection plug were attached to the catheter and after assuring catheter patency, the hub was sutured close to the skin. Bupivacaine 1–2mg/kg was injected around the surgical sites after skin closure. Cotton sleeves covering the neck and shoulder areas were placed to prevent the rabbits from scratching the surgical sites. Pain control was achieved with subcutaneous administration of buprenorphine 0.03–0.05 mg/kg every 6–12 hours at the end of surgery and as needed, along with subcutaneous meloxicam 0.2 mg/kg daily on days 1–2.

Microbiologic culture

Blood cultures were obtained on days 1, 4 and 7. Rabbits were sedated with subcutaneous acepromazine 1 mg/kg and restrained in a metal cage for the procedure. One ml of blood was drawn from an ear artery and from the catheter. For central blood cultures, the lock solution and 0.5 ml of blood were first aspirated and discarded. After drawing 1 ml of blood for culture, the catheter was flushed with 1 ml of HS 100 units/ml and then locked with either NAC or HS lock solutions. Each 1 ml aliquot of blood was inoculated onto a 150 mm blood agar plate and incubated at 37°C overnight after which colonies were counted. For catheter tip cultures, the intravascular 4-cm catheter tip was washed thrice in phosphate buffered saline (PBS), sonicated for 10 min in 1 ml of PBS, and then vortexed for 30 seconds.(7) Hundred µl aliquots of the resulting suspension and subsequent dilutions were cultured on blood agar plates and colonies counted after overnight incubation at 37°C.

Euthanasia

Rabbits were anesthetized with intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg) followed by intravenous pentobarbital (120 mg/kg). Bilateral thoracotomy was performed prior to carcass disposal.

Study Outcomes

In accordance with relevant clinical events, the primary outcome was the occurrence of *S. aureus* peripheral bacteremia after introduction of the catheter lock solution. Secondary outcome was catheter colonization with *S. aureus*.

Results/Discussion

The culture results of individual rabbits are detailed in Table 1. Prior to NAC or control lock solution placement, all rabbits had negative peripheral blood cultures and all centrally drawn

blood cultures were positive for *S. aureus*. None of the 8 rabbits that received NAC lock solution developed peripheral bacteremia during the study period, whereas 4/7 controls receiving control lock solution did ($p=0.026$; Fisher's exact test). Mean colony counts of *S. aureus* on the catheter tip, measured via sonication, were lower in the NAC-treated vs. control rabbits though this was not statistically significant: $6.09 (\pm \text{SEM } 2.5) \log_{10} \text{ CFU}$ vs. $6.94 (\pm \text{SEM } 2.59) \log_{10} \text{ CFU}$ ($p=0.1$, student's t-test).

Staphylococci are a common cause of nosocomial bloodstream infections and *S. aureus*, in particular, is associated with a high rate of morbidity and mortality.(10) We used *S. aureus* in this experimental model due to its clinical significance, especially in the hemodialysis population.(11) We confirmed that NAC lock solution can effectively reduce the rate of *S. aureus* CAB in vivo. The decrease in bacteremia may be attributed to NAC's bactericidal activity against planktonic organisms released from the biofilm and/or anti-biofilm activity against biofilm present on the luminal aspect of the catheters.(7, 8) The dose of the NAC lock is greater than the minimum bactericidal concentration of NAC against *S. aureus* which is 10 mg/ml.(8)

Since sonication of the catheter tip retrieves bacteria from both the inner and outer surfaces, it may not adequately reflect a decrease in the luminal biofilm burden. This may explain the lack of a significant decrease in numbers of bacterial colonies retrieved from catheter tips in experimental vs. control rabbits. The fact that the bacterial inoculum size was higher than would be expected in clinical practice explains the high bacterial load retrieved from cultured catheters, and this may have played a role in the lack of catheter sterilization.(10) Changing the lock solution every day may potentially be more effective in decreasing biofilm burden than the 72-hour dwell time that was used in this study (to simulate hemodialysis patients). Continuing lock therapy beyond 6 days or increasing the concentration of NAC in the lock solution could have been more protective as well.

All catheters were occluded by day 7 and several catheters were noted to be tied very tightly to the underlying vein at the time of removal. *Staphylococcus aureus* has well known adherence factors for fibrin, fibronectin, and platelets. The production of coagulase may further promote thrombosis as well. Despite rinsing the catheters prior to sonication, there may be non-occlusive thrombi within the catheters containing *S. aureus*. This may be an additional reason for lack of catheter sterilization. There was no difference in catheter occlusion between the 2 groups. In vitro unpublished data indicate that NAC does not interfere with the anticoagulant activity of heparin.

There are some study limitations. First, we instilled the lock solution after introducing the inoculum into the catheter, whereas in a clinical scenario the lock solution is instilled after insertion of the catheter. However, we selected this current experimental model since we were able to demonstrate efficacy of the NAC lock using a relatively small number of rabbits. Second, we inoculated the catheters with a higher inoculum of *S. aureus* than what is seen in clinical practice. Finally, technical aspects of the surgery may have contributed to higher occlusion rate of catheter.

In conclusion, this study assessed the use of a novel non-antibiotic agent as a catheter lock solution for the prevention of catheter-associated bacteremia in an animal model. The results of the study reflect a new and promising facet of N-acetylcysteine in the arena of infection prevention. Since our approach is not antibiotic based, the use of NAC as a lock solution would make it more attractive to use in a clinical population as opposed to antibiotic lock solutions.

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Figure 1.
Study time-line

Table 1

Results of microbiological cultures in individual rabbits in the experimental and control arms.

NAC LOCK	Day 1 peripheral	Day 1 CVC (log10 CFU)	Day 4 Peripheral (log10 CFU)	Day 4 CVC (log10 CFU)	Day 7 Peripheral (log10 CFU)	Day 7 CVC (log10 CFU)	Catheter tip (log10 CFU)
1	NG	4	NG	-	NG	-	6.42
2	NG	>5	NG	-	NG	-	5.75
3	NG	-	NG	-	NG	-	7.1
4	NG	>5	NG	-	NG	-	7.41
5	NG	>5	NG	-	NG	-	7.54
6	NG	>5	NG	-	NG	-	4.67
7	NG	-	NG	-	NG	-	5.52
8	NG	4	NG	-	NG	-	4.3
CONTROLS							
1.	NG	>5	3.08	-	1.45	-	7
2	NG	>5	2.61	>5	0.48	-	7
3	NG	>5	3.06	>5	1.48	-	7
4	NG	>5	3.15	-	2.08	-	7
5	NG	-	NG	-	NG	-	7.04
6	NG	4	NG	-	NG	-	6.15
7	NG	5	NG	-	NG	-	7.4

CVC is central venous catheter. NAC is N-acetylcysteine. NG is no growth. CFU is colony forming unit. - denotes that we were unable to draw blood.