Antibiotic–Heparin Lock: In Vitro Confirmation of Antibacterial Activity

Lavern M. Vercaigne and George G. Zhanel

ABSTRACT

Objective: To determine whether high concentrations of antibiotic "locked" into a central venous catheter with heparin would retain sufficient antimicrobial activity to inhibit the growth of organisms commonly causing catheter-related infections in patients undergoing hemodialysis.

Methods: Cefazolin, vancomycin, ceftazidime (all at final concentrations of 10 mg/mL), and gentamicin (final concentration 5 mg/mL) were "locked" separately with heparin sodium (final concentration 5000 IU/mL) in central venous catheters. The catheters were incubated for 72 h at 37°C in a dark incubator. The solutions were then drained from the catheters and used for microbiological testing against organisms commonly causing catheter-related infections.

Results: In all cases, the antibiotic–heparin solutions drained from central venous catheters after 72 h at 37°C produced zones of inhibition that were not different from those produced by antibiotic controls (at 5 mg/mL).

Conclusions: Each of cefazolin, vancomycin, ceftazidime, and gentamicin locked into central venous catheters with heparin sodium had the ability to inhibit the growth of microorganisms commonly causing catheter-related infections and should be suitable for use in an antibiotic–heparin lock for further in vitro and prospective clinical trials.

Key words: hemodialysis, catheters, bacteremia, antibiotic–heparin lock, in vitro studies

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RÉSUMÉ

Objectif : Déterminer si l'usage de fortes concentrations d'antibiotiques avec de l'héparine, comme «barrière» dans un cathéter veineux central, pourraient maintenir une activité antimicrobienne suffisante pour inhiber la croissance des microorganismes qui sont la cause fréquente d'infections liées au cathéter chez les patients hémodialysés.

Méthode : De la céfazoline, de la vancomycine, de la ceftazidime (toutes aux concentrations finales de 10 mg/mL), ou de la gentamicine (à la concentration finale de 5 mg/mL) a été injectée avec de l'héparine sodique (concentration finale de 5000 UI/mL) comme barrière antimicrobienne des cathéters veineux centraux. Les cathéters ont ensuite été incubés à la noirceur pendant 72 heures à 37 °C. Chacune des solutions injectées dans les cathéters a été par la suite évacuée et soumise à une épreuve antimicrobienne à l'égard des microorganismes qui sont la cause fréquente d'infections dues aux cathéters.

Résultats : Dans tous les cas, les solutions antibiotique–héparine évacuées des cathéters veineux centraux après incubation de 72 heures à 37 °C ont produit des zones d'inhibition qui n'étaient pas différentes de celles produites par les antibiotiques témoins positifs (à 5 mg/mL).

Conclusions : La céfazoline, la vancomycine, la ceftazidime et la gentamicine injectées avec de l'héparine sodique pour servir de barrière antimicrobienne aux cathéters veineux centraux avaient toutes la capacité d'inhiber la croissance des microorganismes qui causent les infections liées au cathéter. Par conséquent, leur usage dans un système de barrière antibiotique–héparine devrait bien se prêter à des études in vitro et à des études cliniques prospectives plus poussées.

Mots clés : hémodialyse, cathéters, bactériémie, système de barrière antibiotique–héparine, études in vitro

INTRODUCTION

Bacteremia is a serious complication of long-term hemodialysis.[1–13] This fact was highlighted in a recent study by Marr and colleagues,[9] who found that bacteremia occurred at a rate of 3.9 episodes per 1000 catheter days (95% confidence interval 3.0 to 4.9 episodes per 1000 catheter days) in 102 patients undergoing hemodialysis with dual-lumen cuffed catheters. Serious complications of bacteremia, including osteomyelitis, septic arthritis, and endocarditis, contribute significantly to morbidity in the hemodialysis population.[9,14]

Initial treatment of catheter-related bacteremia involves IV administration of empiric antibiotics with or without removal of the catheter. Without removal of the catheter, the efficacy of systemic treatment alone ranges from 25% to 32%.[8,9,15] Although removing the catheter increases treatment success rates to approximately 85%, it is not always possible to find a suitable replacement vein in patients with limited access sites.[16] Therefore, in an attempt to eradicate bacteremia while salvaging the access site, exchange of the catheter over a guidewire has been investigated. Small case series have documented successful catheter salvage in approximately 80% to 90% of patients, depending on the period of follow-up.[17–19] However, despite these promising results, the exchange procedure incurs significant costs, including surgical time and the cost of replacement catheters.

As an alternative to catheter replacement, the use of an antibiotic–heparin "lock" along with systemic treatment may help to treat infected central venous catheters and prevent recurrence of bacteremia. The antibiotic–heparin lock is prepared by combining the antibiotic with heparin in a 5-mL syringe under sterile conditions. The solution is then infused into each lumen of the catheter (approximate combined volume of 2.46 mL, depending on the catheter used). The caps of the catheter hubs are then tightly secured to "lock" the antibiotic into the lumens of the catheter. Use of an antibiotic lock has already shown promise in the management of infected catheters used for administering total parenteral nutrition.[20,21] In addition, in a small trial of hemodialysis patients receiving a 4-h continuous infusion, an antibiotic lock (vancomycin or ciprofloxacin 100 µg/mL in 5% sodium heparin) successfully eradicated all 13 episodes of catheter-related sepsis.[22]

To further develop the antibiotic–heparin lock, Vercaigne and colleagues[23] recently investigated the in vitro stability of cefazolin, vancomycin, ceftazidime (all at a concentration of 10 mg/mL), and gentamicin (concentration 5 mg/mL) when combined with heparin sodium (final concentration 5000 IU/mL) in central venous hemodialysis catheters over 72 h. The results of this trial indicated significant adsorption of the antibiotics onto the central venous catheters; however, the final concentration of free antibiotic in solution was still approximately 5 mg/mL, which should suffice as an antibiotic lock.[23] These concentrations are significantly higher than those used in a previous study of antibiotic–heparin combinations.[22] Higher concentrations may be necessary to penetrate slime layers on the luminal surface of the catheters and to overcome a diminished antimicrobial effect on bacteria within the slime layer.[24–28]

In this study, to confirm that these antibiotic solutions retain sufficient antimicrobial activity after incubation with heparin in a catheter for 72 h at 37°C, the solutions were tested against organisms commonly causing catheter-related bacteremia in patients undergoing hemodialysis.

METHODS

Reagents and Catheters

Commercial preparations of cefazolin (SmithKline Beecham), vancomycin (Lilly), ceftazidime (Lilly), gentamicin (Hoechst Marion Roussel), and heparin sodium (Leo) were used. Dual-lumen hemodialysis catheters (20-cm straight catheters made of polyurethane) were obtained from Hospal Gambro (Hechingen, Germany).

Microorganisms

Microorganisms causing frequent or severe catheter-related infections were selected for antimicrobial testing. Specifically, *Staphylococcus epidermidis* and *Staphylococcus aureus* cause most cases (more than 65%) of bacteremia in the hemodialysis population.[29–31] Although gram-negative organisms are not commonly responsible for catheter-related bacteremia, they occasionally do cause this type of infection and can produce significant morbidity.[31] Thus, some gram-negative organisms were also investigated. Clinical isolates of methicillin-sensitive and methicillin-resistant *S. epidermidis*, methicillin-sensitive *S. aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were obtained from the Department of Clinical Microbiology at the Health Sciences Centre, Winnipeg, Manitoba.

Table 1. Semiquantitative Sensitivity (as Zones of Inhibition) of *Staphylococcus epidermidis* and *Staphylococcus aureus* to Cefazolin–Heparin Lock

	Sensitivity of Isolate, as Zone of Inhibition (mm Diameter)		
Test Solution	**MR *S. epidermidis***	**MS *S. epidermidis***	**MS *S. aureus***
Controls			
Cefazolin 5 mg/mL	30	>31	30
Heparin 5000 IU/mL	No inhibition	No inhibition	No inhibition
Combination			
(cefazolin 10 mg/mL* + heparin 5000 IU/mL)			
Catheter 1	30	>31	31
Catheter 2	31	>31	30
Catheter 3	31	>31	30

MR = methicillin-resistant, MS = methicillin-sensitive.
*Original concentration of antibiotic "locked" into the catheter.

Table 2. Semiquantitative Sensitivity (as Zones of Inhibition) of *Staphylococcus epidermidis* and *Staphylococcus aureus* to Vancomycin–Heparin Lock

	Sensitivity of Isolate, as Zone of Inhibition (mm Diameter)		
Test Solution	**MR *S. epidermidis***	**MS *S. epidermidis***	**MS *S. aureus***
Controls			
Vancomycin 5 mg/mL	30	25	22
Heparin 5000 IU/mL	No inhibition	No inhibition	No inhibition
Combination			
(vancomycin 10 mg/mL* + heparin 5000 IU/mL)			
Catheter 1	30	26	23
Catheter 2	31	28	23
Catheter 3	31	26	23

MR = methicillin-resistant, MS = methicillin-sensitive.
*Original concentration of antibiotic "locked" into the catheter.

Study Protocol

After stability of the antibiotic–heparin solutions was confirmed by spectrophotometric and high-performance liquid chromatographic analysis,[23] fresh solutions of cefazolin, vancomycin, ceftazidime (all at final concentrations of 10 mg/mL), and gentamicin (final concentration 5 mg/mL) with heparin sodium (final concentration 5000 IU/mL) were locked in dual-lumen central venous catheters. The combined catheter volume for both lumens was 2.46 mL. The catheters (3 for each antibiotic) were incubated for 72 h at 37°C in a dark incubator. Each of the catheters was subsequently drained into a 1.5-mL Eppendorf tube and immediately transported to the Health Sciences Centre for microbiological testing.

A semiquantitative modified agar dilution procedure was used to investigate the susceptibility of test isolates to the antibiotic solutions.[32] Specifically, a standard inoculum of each isolate was prepared in distilled water to the turbidity of a 0.5 Mcfarland standard (approximately 1 x 10^8 colony-forming units/mL), then swabbed onto a Mueller–Hinton agar plate. A 10-µL inoculum spot of each of the following test solutions was applied to the agar surface: positive control (antibiotic alone), negative control (heparin alone), and test sample (antibiotic–heparin solution). The plates were incubated at 35°C for 18 to 24 hours, and zones of inhibition for each test sample were subsequently measured (in millimetres) and compared with the positive and negative controls.

The "positive" control solutions for cefazolin, vancomycin, and ceftazidime were prepared at a concentration of 5 mg/mL. This concentration was based on a previous stability study indicating that as much as 40% of a 10 mg/mL solution is adsorbed to the luminal surface inside the catheter over 72 h.[23] The "positive" gentamicin control was also prepared at 5 mg/mL, as only 8% of the original 5 mg/mL solution in the catheter adsorbs to the luminal surface.[23] The "negative" control was heparin, at a concentration of 5000 IU/mL.

Table 3. Semiquantitative Sensitivity (as Zones of Inhibition) of *Escherichia coli* and *Pseudomonas aeruginosa* to Ceftazidime–Heparin Lock

Test Solution	Sensitivity of Isolate, as Zone of Inhibition (mm Diameter)	
	E. coli	*P. aeruginosa*
Controls		
Ceftazidime 5 mg/mL	35	33
Heparin 5000 IU/mL	No inhibition	No inhibition
Combination (ceftazidime 10 mg/mL* + heparin 5000 IU/mL)		
Catheter 1	35	33
Catheter 2	35	33
Catheter 3	35	33

*Original concentration of antibiotic "locked" into the catheter.

Table 4. Semiquantitative Sensitivity (as Zones of Inhibition) of *Escherichia coli* and *Pseudomonas aeruginosa* to Gentamicin–Heparin Lock

Test Solution	Sensitivity of Isolate, as Zone of Inhibition (mm Diameter)	
	E. coli	*P. aeruginosa*
Controls		
Gentamicin 5 mg/mL	30	25
Heparin 5000 IU/mL	No inhibition	No inhibition
Combination (gentamicin 5 mg/mL* + heparin 5000 IU/mL)		
Catheter 1	29	23
Catheter 2	29	23
Catheter 3	29	23

*Original concentration of antibiotic "locked" into the catheter.

RESULTS

The results of the semiquantitative sensitivity testing are shown in Tables 1 to 4. There was no zone of inhibition for heparin at a concentration of 5000 IU/mL for any of the organisms tested. The antibiotic controls produced large zones of inhibition at a concentration of 5 mg/mL. In all cases, solutions drained from central venous catheters produced zones of inhibition that were not different from those produced by the positive controls. Specifically, the cefazolin–heparin solution produced zones of inhibition not different in diameter from those produced by the cefazolin controls for both methicillin-resistant and methicillin-sensitive *S. epidermidis* (102% and 100% of control, respectively) and for methicillin-sensitive *S. aureus* (101% of control) (Table 1). Similar results were observed for vancomycin (102% of control for methicillin-resistant *S. epidermidis*, 107% of control for methicillin-sensitive *S. epidermidis*, and 105% of control for methicillin-sensitive *S. aureus*) (Table 2).

Ceftazidime–heparin solutions drained from the central venous catheters produced zones of inhibition not different from those produced by ceftazidime controls when tested against *E. coli* (100% of control) and *P. aeruginosa* (100% of control) (Table 3). Similar results were obtained for gentamicin–heparin solutions when tested against both E. coli (97% of control) and *P. aeruginosa* (92% of control) (Table 4).

Thus, in all cases, antibiotic–heparin combinations drained from central venous catheters in vitro after 72 h of incubation at 37°C in the dark produced zones of inhibition that were not different from positive antibiotic controls (5 mg/mL).

DISCUSSION

The antibiotic–heparin lock is of interest in hemodialysis for treating infected central venous catheters without removing the catheter. It was hypothesized that high concentrations of antibiotic (10 mg/mL) locked into the catheter with heparin (final concentration 5000 IU/mL) for 72 h at 37°C would retain sufficient antimicrobial activity to inhibit growth of organisms that commonly cause catheter-related infections in this population. Although there can be up to 40% adsorption of antibiotic to the catheter surface,[23] starting with initial antibiotic concentrations of 5 to 10 mg/mL should ensure sufficient concentrations of free antibiotic inside the central venous catheter to inhibit microorganisms. Heparin is commonly used inside central venous catheters between hemodialysis treatments to prevent clot formation.[33,34]

The results of this in vitro investigation confirm that the antibiotic–heparin solutions retain the ability to inhibit growth of microorganisms commonly causing catheter-related infections, including *S. epidermidis* and *S. aureus*. In all cases, a 10-µL aliquot of the antibiotic–heparin lock solution produced a zone of inhibition that was not different from the corresponding control antibiotic solution alone (at 5 mg/mL).

Interestingly, cefazolin at a concentration of 5 mg/mL (similar to the concentration that would be available inside the catheter during the procedure) produced large zones of inhibition even against methicillin-resistant *S. epidermidis*. Although this study investigated only a small number of isolates, it provides initial evidence that a cefazolin–heparin lock, prepared at an antibiotic concentration of 10 mg/mL and a heparin concentration of 5000 IU/mL, is sufficient to inhibit growth of *S. epidermidis* in vitro, whether the microbial strain is sensitive or resistant to methicillin. Therefore, this may be the preferred combination of agents for further studies examining the efficacy of the antibiotic–heparin lock.

Although this study has shown that the antibiotic–heparin solutions drained from the catheters inhibited growth of the organisms tested, further in vitro studies should investigate the kill curve characteristics of the antibiotic–heparin lock solutions inside central venous catheters inoculated with microorganisms. In addition, the number of isolates tested in this study (5) was small, and the results should be confirmed with a greater number of isolates from documented central venous catheter infections in hemodialysis patients. Prospective clinical trials are also required to confirm these in vitro observations.

In conclusion, cefazolin, vancomycin, ceftazidime (all prepared at a final concentration of 10 mg/mL), and gentamicin (final concentration 5 mg/mL) locked into a central venous catheter at 37°C for 72 h with heparin sodium (final concentration 5000 IU/mL) have the ability to inhibit the growth of microorganisms commonly causing catheter-related infections. These combinations should be suitable as antibiotic–heparin locks for further in vitro and prospective clinical trials.

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