

## Elimination of Intraluminal Colonization by Antibiotic Lock in Catheters

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ONCU, S., ONCU, S., OZTURK, B., KURT, I. and SAKARYA, S. *Elimination of Intraluminal Colonization by Antibiotic Lock in Catheters*. Tohoku J. Exp. Med., 2004, **203** (1), 1-8 — Antibiotic lock (AL) technique for catheter related infection encompasses the filling of a catheter lumen with high concentrations of antibiotics for hours. The goal of AL therapy is to decontaminate the intraluminal surface of the catheter. However the duration of antibiotic therapy is not established. An in vitro model was designed to establish the time needed to eliminate intraluminal microbial colonization and to evaluate the efficacy of vancomycin in comparison with teicoplanin by using laboratory AL model. Human plasma was instilled into the catheters to allow deposition of fibrin and other products on the catheter wall. After 48 hours, the catheters were drained and inoculated with bacteria in tryptic soy broth. The catheters were then drained and filled with either (a) vancomycin saline solution (VSS) lock (b) teicoplanin saline solution (TSS) lock or (c) saline solution (SS) as the control and then incubated for 12 hours. After 12 hours incubation all the catheter were drained and filled with human plasma. Instillation of human plasma and AL was alternated every 12 hours to simulate clinical conditions. For each day three catheters, locked with VSS, TSS and SS were cultured for colony count. Microbial counts were expressed as total colony-forming units per longitudinal centimeters of catheter surface. A significant decrease in intraluminal catheter colonization started as early as day 1. At the end of 7th day catheters treated with VSS and TSS lock were completely sterile. The decrease of intraluminal colonization was similar in catheters treated with VSS and TSS lock. Also the decrease of intraluminal colonization were similar in catheter colonized with slime forming *S. epidermidis* and non-slime-forming *S. epidermidis*. ——— antibiotic lock; catheter; teicoplanin; vancomycin

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Received January 5, 2004; revision accepted for publication February 5, 2004.

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Central venous catheters (CVCs) are used for the monitoring and therapy of critically ill patients (Bouza et al. 2001; Oncu and Sakarya 2003). Estimates of their use in the United States alone suggest that over five million CVCs are inserted annually (Pittet et al. 1995; Oncu et al. 2003). In particular, the use of tunneled Broviac - Hickman-type catheters and totally implantable venous access devices (ports), have increased notably over the past years (Nouwen et al. 1999). Unfortunately, these devices are associated with a number of complications, amongst which infection predominates. CVCs are probably responsible for about 250 000 cases per year of nosocomial bacteremia in the United States, although some estimates are as high as 400 000 cases per year (Fraenkel et al. 2000; O'Grady et al. 2002). Currently, catheter related infection (CRI) is a major cause of patient morbidity and mortality, a reason for premature catheter removal and an explanation for the increase in cost and use of resources (Dimick et al. 2001). The appropriate management of CVCs has therefore, become a major challenge for physicians. Difficult to replace CVC, subcutaneous reservoirs and implantable device associated infections have been successfully treated with the antibiotic lock (AL) technique (Carratala 2002; Viale et al. 2003). The AL technique consists of filling the catheter lumen with an antibiotic solution and allowing it to dwell for a period of time, in order to sterilize the device (Cuntz et al. 2002; Viale et al. 2003). The duration of antibiotic lock therapy is not established, however most studies institute therapy for 10-14 days. The aim of this study was to establish the time needed to eliminate intraluminal microbial colonization and to evaluate the efficacy of vancomycin in comparison with teicoplanin, by using laboratory AL model.

## MATERIALS AND METHODS

### *Bacterial strains*

Both slime-forming *S. epidermidis* and non-slime - forming *S. epidermidis* isolated from CRI were used in the study. Identification of clini-

cal isolates was determined by Gram staining, catalase reaction, tube coagulation test and Api-staph test (biomérieux, Lyon, France). The ability of the *S. epidermidis* strain to form a biofilm on an abiotic surface was determined as described elsewhere (O'Toole and Kolter 1998). Briefly, *S. epidermidis* strains were grown overnight in Tryptic soy broth (TSB) with 0.25% glucose at 35°C. The culture was diluted 1:40 in TSB-0.25% glucose, and 2 ml of this cell suspension was used to inoculate sterile polypropylene conical bottom tubes (TPP, Trasadingen, Switzerland). After 48 hours at 35°C, tubes were gently washed three times with 2 ml of phosphate-buffered saline, dried in an inverted position, and stained with 2 ml of 0.25% safranin for 2 minutes. The tubes were rinsed again, and color development at the inner surface of the tube was observed. A positive result was indicated by the presence of adherent layer of stained material on the inner surface of the tube. Moreover, slime formation of strains was also shown quantitatively with 96-well polystyrene U bottom microtiter plates (TPP, Trasadingen, Switzerland) as described (Toledo-Arana et al. 2001). The optical density of wells content was determined at 595 nm (OD<sub>595</sub>) using a microplate reader (Biotek Instruments, Winooski, VT, USA). Strains with same OD<sub>595</sub> values to baseline were defined as non-slime-forming *S. epidermidis*. *S. epidermidis* with OD<sub>595</sub> values >2 were accepted as slime-forming strain. The MIC value, determined by the NCCLS recommended broth microdilution testing method (NCCLS 2000), of each antibiotic for each isolate was 4 mg/liter.

### *Antimicrobial agents*

Antimicrobial agents used in this study were prepared from their commercially available forms. These were vancomycin (Eli Lilly, Indianapolis, IN, USA) and teicoplanin (Lepetit & Aventis, Levallois Perret, France). Antimicrobial agents were mixed with 0.9% saline and prepared in a concentration of 5 mg/ml.

### Catheters

The catheters used in this study were approximately 4 cm segments of catheters made of polyurethane material (Arrow, Erding, Germany). The catheters were divided into two groups; catheters infected with slime-forming *S. epidermidis* and catheters infected with non-slime forming *S. epidermidis*. A total of 45 catheters were included in each group. In each group, catheters were locked with vancomycin-saline solution (VSS) ( $n=15$ ), teicoplanin-saline solution (TS) ( $n=15$ ) and with saline solution (SS) ( $n=15$ ) as control group.

### Experiments

Using sterile procedures, the catheters were clamped at the distal end and human plasma was instilled into the catheters to allow deposition of fibrin and other products on the CVC wall. After 48 hours, the catheters were drained and inoculated with bacteria in TSB suspension. The inoculated bacteria were harvested from overnight agar cultures and inoculated to TSB to achieve a concentration of  $1.5 \times 10^8$  (0.5 McFarland). After inoculation of the bacteria the catheters were incubated at 35°C for 24 hours. Catheters were then drained and filled with (a) VSS lock, (b) TSS lock or (c) SS solution as the control and then incubated for 12 hours. After 12 hours incubation all

the catheter were drained and filled with human plasma. Instillation of human plasma and AL was alternated every 12 hours to simulate clinical conditions. For each day three catheters, locked with VSS, TSS and SS, from each group were cultured for colony count. A 1-cm catheter segment per sample were flushed with 2 ml TSB solution and vortexed within the solution for 4 minutes in 2500 rpm. TSB solution in which the catheters were vortexed were serially diluted in TSB and plated on blood agar. Inoculated blood agars were incubated at 35°C for 24 hours. Microbial counts were expressed as total colony-forming units per longitudinal centimeters of catheter surface. The study was carried out until the catheters were sterile. Experiments were conducted in triplicate and repeated three times.

### Statistical analysis

Differences in microbial recovery were analyzed by two-sample *t*-test. The cutoff level of significance was a *p*-value of  $< 0.05$ .

## RESULTS

For both isolates, bacterial counts  $>10^7$  were maintained after administration of the saline control lock for 10 days, confirming the viability of *S. epidermidis* inside the catheter. Differences in microbial recovery among the test strains colo-

TABLE 1. Mean Microbial Recovery (cm catheter surface) of Catheters\*

Day	Non slime producing <i>S. epidermidis</i>			Slime producing <i>S. epidermidis</i>		
	SS**	VSS***	TSS†	SS**	VSS***	TSS†
0††	$9 \times 10^6$	$9 \times 10^6$	$9 \times 10^6$	$15 \times 10^6$	$15 \times 10^6$	$15 \times 10^6$
1	$>10^7$	$15 \times 10^5$	$14 \times 10^5$	$>10^7$	$21 \times 10^5$	$19 \times 10^5$
2	$>10^7$	$18 \times 10^4$	$16 \times 10^4$	$>10^7$	$26 \times 10^4$	$22 \times 10^4$
3	$>10^7$	$12 \times 10^3$	$10^4$	$>10^7$	$2 \times 10^4$	$18 \times 10^3$
4	$>10^7$	$10^3$	$9 \times 10^2$	$>10^7$	$16 \times 10^2$	$14 \times 10^2$
5	$>10^7$	80	70	$>10^7$	120	100
6	$>10^7$	8	6	$>10^7$	12	10
7	$>10^7$	-	-	$>10^7$	-	-

\* Statistically significant,  $p < 0.001$ . Significant decrease in microbial recovery started as early as day 1.

\*\* Saline Solution, \*\*\* Vancomycin Saline Solution (5 mg/ml), † Teicoplanin Saline Solution (5 mg/ml),

†† Indicates the starting day of AL therapy

nizing the intraluminal surface demonstrate that organisms vary in their ability to adhere to the catheter surface. Slime forming *S. epidermidis* were shown to adhere in greater numbers ( $15 \times 10^6$  cfu) to catheter segments when compared with non-slime forming *S. epidermidis* ( $9 \times 10^6$  cfu). Bacterial counts after administration of the VSS, TSS and SS locks are shown day by day in Table 1. A significant decrease in intraluminal catheter colonization started as early as day 1 ( $p < 0.001$ ) and attend to decrease in further days of VSS and TSS lock therapy. At the end of 5th day the bacterial count was below the critical level of  $< 10^3$  and at the end of 7th day catheters were completely sterile. The decrease of intraluminal colonization was similar in catheters treated with VSS and TSS lock. Also the decrease of intraluminal colonization were similar in catheter colonized with slime forming *S. epidermidis* and non-slime-forming *S. epidermidis*.

After 7th day catheters were instilled only with human plasma for 24 hours and cultured upto 10th day to show total clearance of bacteria. There was no bacteria isolated after 7th day in the catheters treated with VSS and TSS.

## DISCUSSION

Intravascular catheter related infections are a major cause of morbidity and mortality (Elliott 2001). These type of infections present a challenge because of the difficulty that can had with treatment. Although catheter removal is frequently viewed as the only appropriate treatment, this procedure raises important practical problems in "highly needed" tunneled catheters (Carratala 2002). These catheters are expensive and are used in patients who are frequently debilitated and with no other available vascular access. In these clinical settings, an attempt to treat catheter-related bacteremia is often carried out with a seven to 21 day course of systemic antibiotics administered through the catheter without catheter removal. However, this approach fails in approximately 30% of treatments, resulting in removing of the device (Groeger et al. 1993; Rotstein et al.

1995; Carratala 2002). This failures is explained by the fact that most antibiotics cannot kill microorganisms growing on the biofilm that lines catheter lumens with therapeutic levels of antibiotics (Carratala 2002). To overcome these problems, and taking into consideration the fact that majority infections in tunneled catheters originated in the catheter hub and spread intraluminally, an AL technique has been developed for treatment of CRI. The AL technique encompasses the filling of a catheter lumen with high concentrations of antibiotics for a period of time, in order to sterilize the device. With this method, a high local concentration of an appropriate antibiotic can be applied in the catheter lumen while avoiding systemic toxicity and the need to monitor serum drug levels (Mermel et al. 2001; Carratala 2002). This study examined the effect of selected antibiotics on intraluminal colonization and the sterilization time of the catheters with AL technique.

Several open trials of antibiotic lock therapy of tunneled catheter-related bacteremia, with or without concomitant parenteral therapy, have reported a response and catheter salvage without relapse in 138 (82.6%) of 167 episodes (Messing et al. 1988; Capdevila et al. 1993; Johnson et al. 1994; Williams et al. 1994; Benoit et al. 1995; Krzywda et al. 1995; Mermel et al. 2001). Compared with parenteral therapy used in the aforementioned open trials, therapy including AL was significantly more likely to result in catheter salvage. Although the duration of AL therapy has varied among these studies, it most often was 2 weeks. In case there is no indication for catheter removal, the current recommendation for the treatment of tunneled CVC – or implantable device related bacteremia due to *S. epidermidis* and other bacteria is systemic antibiotic therapy and AL therapy (Mermel et al. 2001). It is also possible to treat CRI due to *S. epidermidis* in removable CVC related bloodstream infections without catheter removal (Mermel et al. 2001). For both cases the recommended time for AL therapy is 14 days (Mermel et al. 2001). In our study there was steady decrease in the bacterial count after start-

ing AL therapy. The bacterial count decreased to a critical level,  $<10^3$  cfu, after the 5th day of therapy and the catheters were completely sterile at the end of the 7th day. The duration needed to sterilize the catheter was the half time of the current recommendation for AL therapy. One in vitro AL study, utilized 7 different antibiotics including vancomycin against various strains of isolated microorganisms. Vancomycin, 83 mg/ml, was tested in catheters infected with both slime-forming and non-slime forming *S. epidermidis*. The antibiotics were instilled in a catheter 12 hours daily for 7 days, while parenteral nutrition therapy was instilled in the infected catheter for the remaining 12 hours each day. Intraluminal colonization was eliminated at the 4th day of therapy ( $p<0.001$ ) (Andris et al. 1998). Another in vitro study evaluated the efficacy of conventional and non-conventional 3 day therapy involving vancomycin for treating catheters colonized with *S. epidermidis*. The study evaluated the efficacy of vancomycin infused intermittently every 8 hours, continuously over 24 hours, and via antibiotic lock, in 5 catheters. The antibiotic dwell time for the antibiotic lock was 24 hours with vancomycin (5 mg/ml), in which the solution was changed every 12 hours. The investigators found that antibiotic lock therapy sterilized all 5 catheters, whereas the intermittent and continuous infusions of vancomycin alone did not sterilize any of the catheters. This in vitro study demonstrated greater efficacy with antibiotic lock therapy compared with continuous or intermittent infusions of vancomycin (Gaillard et al. 1990). In another study, vancomycin lock solution (5 mg/ml) was tested against catheters infected with *S. epidermidis*. The catheters were locked with vancomycin for 48 hours in the lumen of the catheter. At the end of the therapy the growth of the organism was reduced by  $>96\%$  (Vercaigne et al. 2002). All of these in vitro studies support our findings that the catheters are decontaminated within a couple of days after AL therapy. The variation of time to sterilize the catheters may be the result of different dosages and dwelling time of vancomycin

used in these in vitro studies. In clinical practice glycopeptide antibiotics are mostly used at the concentration of 5 mg/ml and dwelled in the catheter lumen for 12 hours. For this reason we conducted this study with this dosage and dosing intervals.

Clinical trials are missing regarding to treat catheters with AL therapy for shorter time. In most of the studies the duration of therapy was often 2 weeks. But in a study with the lowest salvage rate reported that a mean of 8 days of therapy was sufficient. This clinical study also supports our findings (Krzywda et al. 1995).

It is known that unnecessary use of glycopeptide antibiotics contributes to the development of glycopeptide resistant Gram-positive organisms (Sieradzki et al. 1999; Carratala 2002; Oncu et al. 2004). Infections due to these resistant organisms are difficult to treat, and there is increased morbidity and mortality with these type of infections. One of the way to prevent the emergence of these resistant organisms is to use antibiotics more carefully. According to our study glycopeptide antibiotics should be used for shorter time for AL therapy.

Many microorganisms in the presence of a biomaterial will adhere tenaciously to the inert surface. Studies of microbial adherence to biomaterials have demonstrated that once an organism adheres to the surface of a biomaterial, its antimicrobial susceptibility is altered such that the MIC often shifts toward a resistant range (Gristina et al. 1989). Coagulase-negative staphylococci have emerged as an important group of organisms in CRI, with *S. epidermidis* being the most predominant (Sherertz et al. 1990; Huebner and Goldmann 1999). The coagulase-negative staphylococci are capable of producing an exopolysaccharide substance (slime) that mediates microbial adherence to biomaterial surfaces and prevents penetration of the antibiotic into the bacterial cell (Gristina et al. 1989; Boussard et al. 1993; Donelli et al. 2001). In antibiotic lock therapy very high concentrations of antibiotics are used, and we inquired if these high concentrations of antibiotics may

overcome the effect of the slime. For this reason, to compare the effect of high dose of antibiotics on slime forming and non-forming isolates, we divided the catheters in to two groups. According to our results, slime forming *S. epidermidis* were as successfully treated as was non-slime forming *S. epidermidis*. This demonstrates that contact with a highly concentrated anti-infective solution within the catheter lumen is effective in eliminating adherent organisms whether forming slime or not.

Vancomycin and teicoplanin are glycopeptide antibiotics with similar activities. They are used primarily for the treatment of Gram-positive infections. There is no comparative study of vancomycin and teicoplanin in the literature regarding AL therapy. No significant differences in growth reduction were observed between the vancomycin and teicoplanin containing antibiotic-saline locks. There are many studies in which vancomycin was evaluated for the AL technique (Gaillard et al. 1990; Benoit et al. 1995; Andris et al. 1998; Carratala et al. 1999; Haimi-Cohen et al. 2001; Vercaigne et al. 2002). But studies with teicoplanin are limited. Some studies support the effectiveness of teicoplanin as lock therapy but studies conducted by Longuet et al. (1995) and Guedon et al. (2002) showed limited efficacy with teicoplanin lock therapy (Longuet et al. 1995; Bregenzer and Widmer 1996; Cuntz et al. 2002; Guedon et al. 2002). According to our study the efficacy of both vancomycin and teicoplanin lock solutions were similar and both antibiotics may be selected for AL therapy in CRI due to methicillin resistant Staphylococci.

In summary, our study indicate that AL therapy with vancomycin and teicoplanin in a concentration of 5 mg/ml are similarly effective and absolutely clear catheters colonized with *S. epidermidis* in 7 days in vitro. But further prospective, randomized clinical trials are required for to determine the in vivo relevance of these findings.

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