ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2004, p. 1727–1732 Vol. 48, No. 5
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.5.1727–1732.2004

Rabbit Model of *Candida albicans* Biofilm Infection: Liposomal Amphotericin B Antifungal Lock Therapy

Matthew K. Schinabeck,[1,2] Lisa A. Long,[1] Mohammad A. Hossain,[1] Jyotsna Chandra,[1] Pranab K. Mukherjee,[1] Sotohy Mohamed,[1] and Mahmoud A. Ghannoum[1]*

Center for Medical Mycology and Mycology Reference Laboratory, Department of Dermatology,[1] and Division of Infectious Diseases, Department of Medicine,[2] University Hospitals of Cleveland and Case Western Reserve University, Cleveland, Ohio 44106

Received 23 December 2003/Returned for modification 6 January 2004/Accepted 14 January 2004

Catheter-related infections due to *Candida albicans* biofilms are a leading cause of fungal nosocomial bloodstream infection. In this paper, we describe the development of a model of catheter-associated infection with *C. albicans* biofilms and show that antifungal lock therapy with liposomal amphotericin B is an effective treatment strategy for these infections. Silicone catheters surgically placed in New Zealand White rabbits were infected with *C. albicans*, and the rabbits were randomized into three groups: (i) untreated controls, (ii) liposomal amphotericin B lock, and (iii) fluconazole lock. Upon completion of therapy, blood cultures were obtained and the catheters were removed for quantitative culture and scanning electron microscopic analyses. Quantitative cultures revealed that catheters treated with liposomal amphotericin B yielded 0 CFU, which was significant compared to the untreated controls ($P < 0.001$) and the fluconazole-treated group ($P = 0.0079$). Although fluconazole treatment tended to have lower CFU compared to untreated controls, there was no difference in mean colony counts between these two groups (1.128 ± 0.764 and 1.841 ± 1.141 $\log_{10}$ CFU/catheter segment, respectively; $P = 0.297$). Scanning electron microscopy revealed abundant biofilm in the control and fluconazole groups, while the liposomal amphotericin B group was virtually cleared. These findings suggest a possible treatment strategy for the successful salvage of catheters infected with *C. albicans* biofilms and describe an animal model that may play an important role in the further study of *C. albicans* biofilm pathogenesis and evaluation of potential antibiofilm agents.

Nosocomial bloodstream infections are a significant cause of morbidity and mortality among hospitalized patients, with *Candida* species representing the fourth most common cause of such infections (9). Central venous catheters (CVCs) are responsible for many of these infections, and up to 40% of patients with *Candida* isolated from their CVCs have underlying fungemia (1). Despite the introduction of new antifungal therapies, the attributable mortality of patients with candidemia remains unacceptably high, ranging from 39 to 49% (10, 26).

Adherence of microorganisms and biofilm formation on catheter surfaces is a common mechanism for catheter-associated bloodstream infection (20). These biofilms are characterized by the production of a thick extracellular matrix and an altered resistance phenotype (8). Electron microscopic evaluation of catheters removed from patients has demonstrated widespread biofilm colonization, confirming the importance of biofilms in catheter-associated infections (20). CVCs infected with *Candida* biofilms are especially problematic since *Candida* species adhere avidly to catheter material and antifungal therapy alone is insufficient for cure (6). This explains the rationale behind the recent national guidelines recommending the removal of catheters infected with *Candida* in order to eradicate a potential nidus of bloodstream infection (17, 19). However, catheter removal is not always feasible for patients with coagulopathy or limited vascular access and is associated with increased healthcare expenses as well as complications related to catheter replacement (18).

Data from several laboratories have demonstrated the near-total resistance of in vitro *Candida albicans* biofilms to common antifungal agents (6, 7, 13, 15). More recently, our group and others have observed that in vitro *C. albicans* biofilms show susceptibility to the lipid formulations of amphotericin B and the echinocandins (3, 16). Although these data suggest a possible strategy for the salvage of CVCs infected with *C. albicans* biofilms, the clinical significance of these observations remains unknown. There has been no in vivo validation of these findings because (i) no animal model of catheter-related *C. albicans* biofilm infection is available and (ii) no prospective, randomized clinical trials have been performed. In this study, we describe the development of the first clinically relevant animal model of *C. albicans* biofilm-associated catheter infection and evaluate the effectiveness of liposomal amphotericin B antifungal lock therapy.

MATERIALS AND METHODS

Model development. (i) Catheter placement. All animal experimentation was performed in accordance with the guidelines for animal health and welfare required by the Institutional Animal Care and Use Committee at Case Western Reserve University School of Medicine, Cleveland, Ohio. Surgical placement of the CVCs was modified from previously described methods (11, 25). Female New Zealand White rabbits weighing 2.5 to 3.0 kg (Covance Inc., Kalamazoo, Mich.) were used for all procedures. Silastic tubing, 0.04-in. internal diameter × 0.085-in. external diameter (Dow Corning, Midland, Mich.), was precut to 30 cm, and a polyethylene cuff (PE 240; Becton Dickinson, Sparks, Md.) was slipped over the

* Corresponding author. Mailing address: Center for Medical Mycology, Department of Dermatology, University Hospitals of Cleveland and Case Western Reserve University, 11100 Euclid Ave., Cleveland, OH 44106-5028. Phone: (216) 844-8580. Fax: (216) 844-1076. E-mail: mag3@cwru.edu.

catheter 4 cm from one end and superglued in position. The purpose of the cuff was twofold: (i) it marked how far to insert the catheter, thus preventing damage to the tricuspid valve and possible endocarditis, and (ii) it protected the catheter from collapsing when the distal suture was tied. Polyurethane catheters (5 French) were purchased commercially (Instech Solomon, Plymouth Meeting, Pa.).

The tubing was sterilized by ethylene oxide. Rabbits were given intramuscular anesthesia with ketamine (70 mg/kg of body weight) and xylazine (7 mg/kg of body weight). The right cervical, shoulder, and scapular regions were shaved with electric clippers, and the area was washed with betadine soap followed by isopropyl alcohol and betadine solution. A 4-cm incision was made in the right anterolateral cervical region, exposing the external jugular vein. A segment of the external jugular vein, just distal to the bifurcation of the internal and external maxillary veins, was freed from subcutaneous fat, and an 18-in. × 1/4-in. Penrose drain (Medline Industries, Inc., Mundelein, Ill.) was placed under the vein (Fig. 1A). Two segments of 3-0 vicryl suture (Ethicon Inc., Somerville, N.J.) were placed proximally and distally to the Penrose drain. The catheter was flushed with sterile saline and clamped with a hemostat. Using a no. 11 scalpel blade, an incision in the isolated segment of vein was made, and bleeding was controlled with upward traction of the Penrose drain. The catheter was inserted into the vein caudally 4 to 5 cm, up to the cuff (Fig. 1B), placing the catheter tip in the right anterior vena cava as demonstrated in the venogram in Fig. 1G. The proximal and distal ligatures were tied, and blood was withdrawn to test catheter patency (Fig. 1C). The catheter was then flushed with heparinized saline. Passing a hemostat cephalad through a 1.0-cm incision in the intrascapular region to the external jugular vein incision site created a subcutaneous tunnel. The hemostat was used to pull the catheter through the subcutaneous tunnel, the excess catheter was cut, and an 18-gauge Luer stub adapter (Becton Dickinson) and a sterile heparin lock device (Medex, Hilliard, Ohio) were placed on the proximal end. Catheter patency was tested again by withdrawing blood and flushing the catheter with heparinized saline. The neck incision was closed by using 3-0 vicryl sutures. The Luer hub was buried in the subcutaneous tract, and a purse string suture was used to attach the heparin lock device flush to the skin (Fig. 1D to F). For the first three postoperative days, the animals received buprenorphine for postsurgical pain control and penicillin G and gentamicin to prevent surgical site infection.

(ii) Inoculation of catheter. The *C. albicans* isolate (M61) used in this study was obtained from a CVC tip of a patient with catheter-associated candidiasis as previously described (16). The mean inhibitory concentrations of liposomal amphotericin B and fluconazole against this strain in the planktonic and biofilm form were 0.5 and 0.25 μg/ml (planktonic) and 1 and >256 μg/ml (biofilm), respectively. *C. albicans* M61 cells were grown, harvested, washed, and counted as previously described (6). A standard 300-μl inoculum consisting of 10^7 CFU of *C. albicans*, 100 U of heparin (Abbott Laboratories, North Chicago, Ill.), and sterile normal saline was prepared. After catheter placement, the inoculum was "locked" in the internal lumen of the catheter and allowed to dwell for 24 h. The inoculum was removed, and the catheter was flushed daily with 300 μl of heparinized saline (100 U).

(iii) Catheter removal. Seven days postinfection, the rabbits were anesthetized as described above, and 5 ml of blood was obtained for blood culture (BD Bactec, Becton Dickinson) through the catheter and via a cardiac puncture. The animals were euthanized with an intracardiac injection of pentobarbital. By using sterile technique, the catheter was removed and divided into proximal (subcutaneous tunnel) and distal (intravenous) 4-cm segments. Each segment was divided in half (2 cm) for (i) quantitative culture and (ii) examination by scanning electron microscopy (SEM).

(iv) Quantitative catheter culture. Using sterile technique, catheter segments were cut in half lengthwise and placed in 10 ml of sterile saline. Specimens were sonicated at 40,000 Hz (Bransonic 1510; Branson Ultrasonics Corp., Danbury, Conn.) for 12 min at 4-min intervals and then vortexed for 15 s. Serial dilutions were performed prior to plating 1-ml aliquots onto Sabouraud dextrose agar (Difco Laboratories) supplemented with chloramphenicol and gentamicin. The plates were incubated for 48 h at 37°C prior to counting CFU. Based on prior clinical studies, catheters growing 10^2 CFU or more of *C. albicans* were considered infected (17).

(v) SEM of catheter segments. Catheter segments were fixed in 2% glutaraldehyde and prepared for SEM observation according to previously published protocol (7). The fixed and dehydrated catheter segments were sputter coated with gold-paladium (60/40) and viewed under a Phillips XL30 scanning electron microscope.

Antifungal lock experiments. (i) Antifungals. Liposomal amphotericin B was obtained from Fujisawa Healthcare, Inc., and Gilead Sciences (San Dimas, Calif.). Fluconazole was provided by Pfizer Pharmaceuticals Group (New York, N.Y.). Liposomal amphotericin B was reconstituted in sterile water and diluted with 5% sterile dextrose, while the fluconazole was reconstituted and diluted with sterile water.

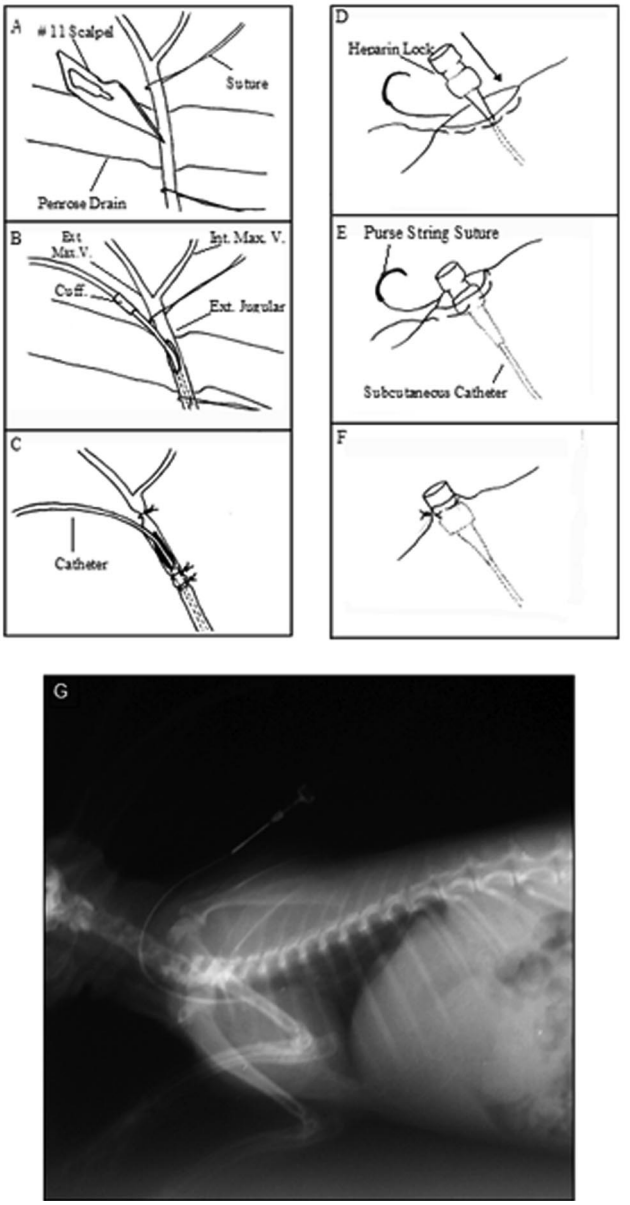

FIG. 1. Surgical placement of the intravenous catheter. (A to C) Catheter insertion into the external jugular vein; (D to F) attachment of the heparin lock device to skin; (G) postoperative venogram of catheter placement.

(ii) Catheter treatment. Each catheter was flushed with 300 μl of heparinized saline (100 U) on a daily basis for the first three days of the study. On day 3 postinfection, 5 ml of blood was drawn through each catheter for culture. To determine the efficacy of antifungal lock therapy, the rabbits were randomized into three groups, each consisting of seven animals. Group I received daily catheter flushes with 300 μl of heparinized (100 U) saline. Catheter lumens of animals in group II were locked with a 300-μl solution containing (i) 3 mg of liposomal amphotericin B, (ii) 100 U of heparin, and (iii) 5% dextrose solution. Rabbits in group III had their catheter lumens locked with a 300-μl solution containing (i) 3 mg of fluconazole, (ii) 100 U of heparin, and (iii) sterile normal saline. The concentration of liposomal amphotericin B used in this study was guided by dosages in prior case reports (4, 14, 24) The fluconazole concentration

was based on one in vitro study (2) and was limited by solubility. The fluconazole dose used in this study required a 10-min incubation in a hot water bath to completely dissolve. The antifungal solutions were locked in the lumen of each catheter for 8 h per day for 7 days. Upon completion of each daily treatment, the antifungal lock solution was removed and the catheter was flushed with 300 μl of heparinized saline. If the antifungal solution could not be withdrawn, it was flushed into the systemic circulation. After 7 days of antifungal lock therapy, the animals were anesthetized, blood was obtained through the catheter and via a cardiac puncture, and the animals were sacrificed with an intracardiac injection of pentobarbital. The catheters were removed under sterile conditions, and quantitative catheter cultures were performed as described above. Catheters from three animals in each group were examined by SEM.

(iii) Statistical evaluation. The mean CFU from quantitative catheter cultures were compared by the Student's *t* test using StatView version 5.0.1 software (SAS Institute, Cary, N.C.). A P value of < 0.05 was considered significant.

RESULTS

Model development. During the initial development of the animal model, experiments were conducted with both silicone and polyurethane catheters in order to determine if there was a difference in *C. albicans* biofilm formation based on catheter substrate. Seven days postinfection, quantitative catheter cultures consistently yielded greater than 2 $\log_{10}$ CFU/catheter segment, which is considered the threshold for catheter-related infections (17). There was no significant difference between the numbers of CFU found on silicone catheters (3.537 ± 0.752 $\log_{10}$ CFU/catheter segment) and polyurethane catheters (3.286 ± 1.123 $\log_{10}$ CFU/catheter segment; $P = 0.45$). Quantitative cultures of the proximal versus distal catheter segments for both silicone (3.555 ± 0.323 versus 3.519 ± 1.068 $\log_{10}$ CFU/catheter segment; $P = 0.9$) and polyurethane catheters (3.459 ± 0.639 versus 3.114 ± 1.567 $\log_{10}$ CFU/catheter segment; $P = 0.7$) revealed no significant difference. SEM analyses of intraluminal catheter segments consistently revealed the same biofilm architecture regardless of the catheter material used (data not shown). Therefore, we selected silicone elastomer catheters for use in this in vivo model, allowing us to maintain consistency with our in vitro model in which *C. albicans* biofilms are grown on silicone elastomer disks.

Previous in vitro experiments by our group have shown that mature *C. albicans* biofilms form within 48 to 72 h (6). In order to determine if in vivo biofilm formation might occur sooner than 7 days, as initially described, we used quantitative catheter culture and SEM analyses to examine biofilm formation in three animals 72 h postinfection. Mean CFU counts revealed no significant difference between biofilms grown on silicone catheters at 3 versus 7 days for either the proximal (2.257 ± 0.125 versus 3.555 ± 0.323 $\log_{10}$ CFU/catheter segment; $P = 0.705$) or distal segments (2.617 ± 0.221 versus 3.519 ± 1.068 $\log_{10}$ CFU/catheter segment; $P = 0.884$). SEM analyses of infected catheter segments at 3 and 7 days revealed similar architecture consisting of patchy regions of biofilm adherent to the catheter surface, consisting of yeast blastospores encased in thick extracellular material (Fig. 2). Based on these data, the optimal time course of 3 days was selected for mature biofilm formation in subsequent experiments.

Antifungal lock experiments. To confirm the presence of catheter-associated infection prior to the introduction of antifungal lock therapy, blood cultures were drawn through the catheters 3 days postinfection. *C. albicans* was present in 67% of all cultures and 100% of cultures drawn from unclotted catheters (Table 1). Quantitative culture of catheters after 7 days of liposomal amphotericin B lock therapy yielded 0 CFU (Table 1). This finding was significant compared to the untreated controls ($P < 0.001$) and the fluconazole-treated group ($P = 0.0079$). Although fluconazole sterilized both proximal and distal catheter segments in two animals after 7 days of treatment, overall there was no significant difference in mean CFU between the animals treated with fluconazole (1.128 ± 0.764 $\log_{10}$ CFU/catheter segment) and the untreated controls (1.841 ± 1.141 $\log_{10}$ CFU/catheter segment; $P = 0.2973$).

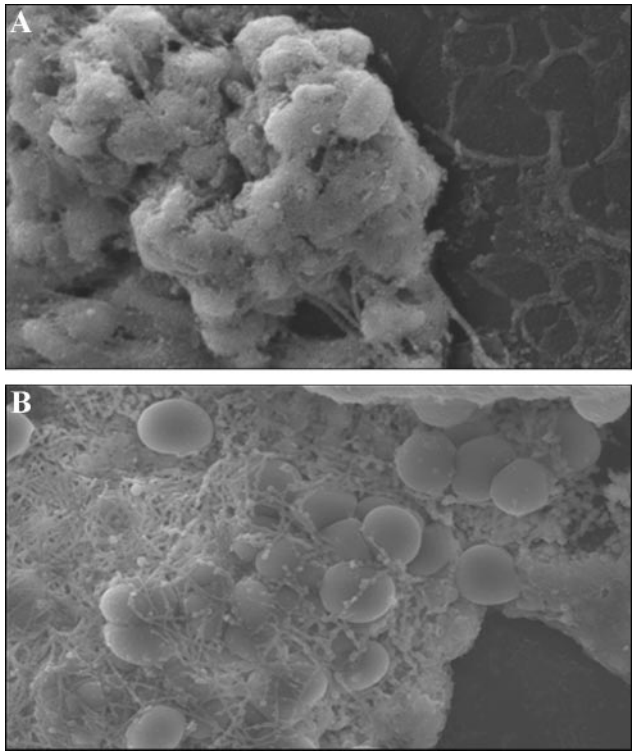

FIG. 2. Mature in vivo biofilm formation during model development. Scanning electron micrographs of *C. albicans* biofilms adherent to the intraluminal surface of catheters showing no difference in biofilm architecture at 7 days postinfection (magnification, ×6,500) (A) and 3 days postinfection (magnification, ×2,500) (B) are shown.

Catheter segments removed from the animals upon completion of treatment were sectioned lengthwise and prepared for SEM examination. In general, the biofilm seen in the untreated controls was patchy in distribution but abundant, consisting of yeast cells encased in a dense extracellular matrix (Fig. 3). Catheters treated with liposomal amphotericin B were completely clear except for 1 to 2 small areas of biofilm per catheter. In these areas, the biofilms seemed to consist of yeast cells that were lacking the thick matrix material evident in the untreated controls. Overall, the fluconazole group had less biofilm than the untreated controls, but patches of yeast cells encased in thick extracellular matrix were readily visible, and the biofilm structure appeared similar to that seen in the untreated controls (Fig. 3).

TABLE 1. Culture results of antifungal lock studies[a]

Animal	Treatment	Blood cultures			Quantitative catheter cultures (log_{10} CFU/cath segment ± SD)	
		Postinfection (day 3) cath	Posttreatment (day 10) cath	Posttreatment (day 10) cardiac puncture	Proximal	Distal
29709	Control	Pos	Pos	Pos	0.894 ± 1.189	0.426 ± 0.00
29711	Control	Clot	Pos	Neg	2.329 ± 0.119	2.872 ± 0.057
41365	Control	Pos	Clot	Neg	2.779 ± 0.111	2.891 ± 0.033
41367	Control	Pos	Clot	Neg	3.794 ± 0.154	3.312 ± 0.036
29797	Control	Clot	Pos	Neg	1.387 ± 0.234	1.466 ± 0.080
29049	Control	Pos	Clot	Pos	1.076 ± 0.357	0.125 ± 0.00
29044	Control	Clot	Clot	Neg	1.418 ± 0.166	1.000 ± 0.00
29710	L-AmB	Pos	Neg	Neg	0	0
29713	L-AmB	Pos	Pos	Neg	0	0
29708	L-AmB	Pos	Clot	Neg	0	0
41369	L-AmB	Clot	Neg	Neg	0	0
41371	L-AmB	Pos	Pos	Neg	0	0
29043	L-AmB	Pos	Neg	Neg	0	0
29045	L-AmB	Clot	Neg	Neg	0	0
29042	Fluc	Pos	Clot	Neg	1.529 ± 0.228	1.316 ± 0.133
29041	Fluc	Pos	Neg	Neg	0	0
29046	Fluc	Pos	Neg	Neg	0	1.023 ± 0.707
29050	Fluc	Clot	Pos	Neg	0	0.813 ± 0.410
41375	Fluc	Pos	Neg	Neg	0	0
41374	Fluc	Clot	Neg	Neg	2.463 ± 0.033	0
41364	Fluc	Pos	Neg	Neg	0.753 ± 0.00	0

[a] L-AmB, liposomal amphotericin B; Fluc, fluconazole; Pos, positive; neg, negative.

DISCUSSION

In this paper, we describe the first clinically relevant animal model of catheter-related *C. albicans* biofilm infection. Using this model, we show that liposomal amphotericin B antifungal lock therapy can sterilize infected catheters. Experiments aimed at optimizing this animal model established by both quantitative culture and SEM analyses that mature biofilms formed equally on polyurethane and silicone CVCs. Previously, Hawser and Douglas reported that *C. albicans* biofilm formation in vitro was significantly decreased when grown on polyurethane compared to silicone elastomer disks (12). Our in vivo studies, however, indicate that there is no difference in *C. albicans* biofilm formation between catheters made of polyurethane and those made of silicone. One explanation for this discrepancy between in vitro and in vivo data may be the formation of conditioning films, or fibrin sheaths, on the intraluminal catheter surface after exposure to blood in vivo. Conditioning films, rich in fibronectin and fibrin, have been shown to coat the surfaces of catheters exposed to blood within hours after catheter insertion (20). Once formed, the conditioning film masks the properties of the underlying catheter material and enhances the adherence of *C. albicans* (8, 21). This finding may explain why there appeared to be no difference between in vivo biofilm formation on silicone and polyurethane catheters.

During the development of this model, we were surprised to find that only 26% of the untreated animals had positive peripheral blood cultures despite documented catheter infection by both quantitative catheter culture and SEM. However, three of five animals with positive peripheral blood cultures had clotted catheters that had been flushed multiple times immediately before the cardiac puncture was performed. The injection of saline through the catheter lumen may have caused a detachment of yeast cells from the catheter-associated biofilms leading to systemic infection. The continuous infusion of fluids through the infected catheters, as routinely occurs in the clinical setting, may lead to a greater percentage of positive peripheral blood cultures. However, in this model, catheters were flushed daily but were otherwise closed to the systemic circulation.

To our knowledge, this study provides the first in vivo SEM images of *C. albicans* biofilms adherent to CVC surfaces. Hawser and Douglas, as well as our group, have previously published in vitro SEM images of *C. albicans* biofilms (7, 12). The dense network of yeast cells, germ tubes, and hyphal forms that comprised the in vitro biofilms are difficult to distinguish in vivo. Instead, the in vivo biofilms were composed of a dense basal layer of yeast cells adherent to the catheter material encased in a thick extracellular matrix. Similar findings have been reported by our group using confocal scanning laser microscopy to examine *C. albicans* biofilms formed on silicone elastomer disks in vitro (6). It is possible that the dense network of yeast cells, germ tubes, and hyphal forms may exist in vivo, but they are not visible due to the limitation of SEM to visualize only the surface topography of the biofilm.

To document the clinical utility of this animal model, we compared the ability of fluconazole and liposomal amphotericin B antifungal lock solutions to sterilize CVCs infected with *C. albicans* biofilms. We selected the antibiotic lock technique because it was developed as a means to overcome the high-grade antimicrobial resistance observed by bacterial biofilms infecting CVCs. By instilling high concentrations of antibiotics

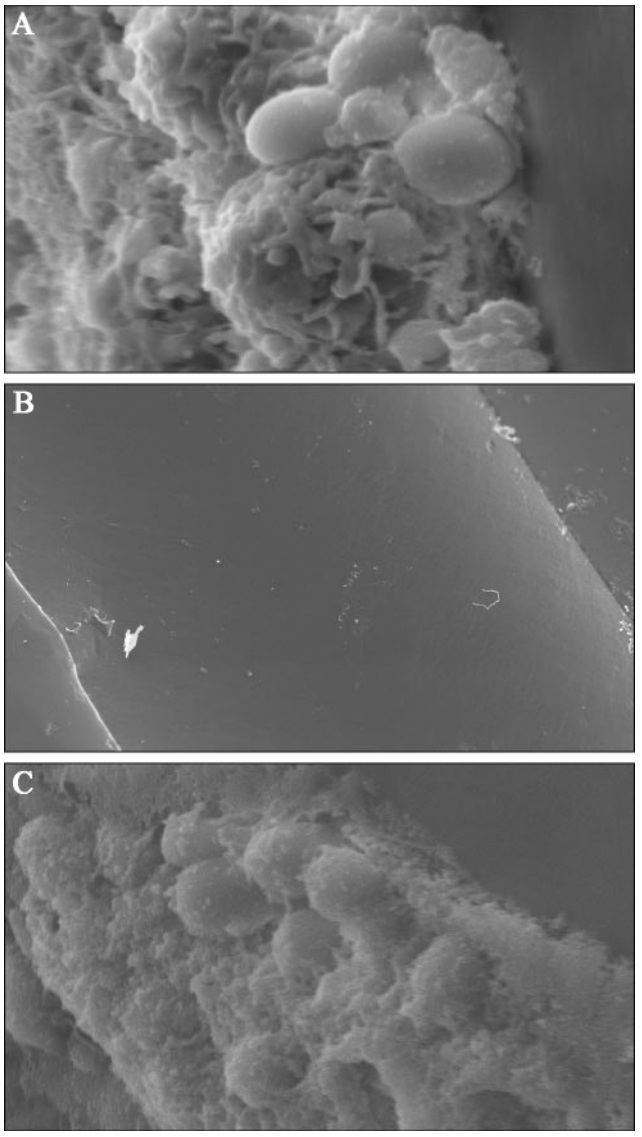

FIG. 3. Effectiveness of antifungal lock therapy. Scanning electron micrographs of intraluminal catheter surfaces following 7 days of therapy with heparinized saline (magnification, ×5,000) (A), liposomal amphotericin B (magnification, ×121) (B), and fluconazole (magnification, ×3,500) (C) are shown.

into the lumen of infected catheters, the antibiotic lock technique is able to (i) overcome the increased resistance of microorganisms in the biofilm state, (ii) avoid systemic drug toxicity and eliminate the need to check drug levels in serum, and (iii) prevent distant spread of microorganisms by closing the catheter during treatment (5). Several open trials of antibiotic lock therapy, with or without concomitant systemic antibiotics, have reported successful catheter salvage without relapse in 83% of those infected with bacteria; however, only 30% of catheters with fungal infections have been successfully retained by using the antibiotic lock technique (17).

Two case reports have documented the successful salvage of catheters infected with *C. albicans* by using amphotericin B deoxycholate antifungal lock therapy. Johnson et al. (14) salvaged two catheters by instilling 3 ml of a 2-mg/ml amphotericin B deoxycholate solution and allowing it to dwell for 12 h per day for 10 to 14 days. A report of the salvage of two more catheters infected with *C. albicans* after treatment with 3 ml of a 2.5-mg/ml amphotericin B deoxycholate solution locked into the catheter lumen for 14 days, with 7 days of concomitant systemic antifungal therapy, was recently published by Viale et al. (24). These results suggest that antifungal lock therapy may be an effective strategy for the retention of catheters infected with *C. albicans.*

The chances of successful catheter salvage may be improved if antifungal agents with enhanced antibiofilm activity are used. Recent in vitro studies performed by our group have identified lipid formulations of amphotericin B and echinocandins as having improved antibiofilm activity compared to other antifungal agents (16). In this study, we validated these findings with our newly developed animal model. Catheters treated with a liposomal amphotericin B antifungal lock solution were completely sterilized, while catheters treated with a fluconazole antifungal lock solution showed no significant difference in *C. albicans* CFU compared to untreated controls.

Suboptimal fluconazole concentrations in the lock solution may explain its inability to sterilize the infected catheters. According to previously published in vitro data, the fluconazole and liposomal amphotericin B MICs for mature biofilms grown with *C. albicans* strain M61 are >256 and 0.25 μg/ml, respectively (16). Therefore, the concentration of liposomal amphotericin B antifungal lock solution used in this study was 12,000 times greater than the MIC for biofilm, while the concentration of fluconazole antifungal lock solution was <11 times the MIC for biofilm. Increased concentrations of fluconazole (300 to 3,000 mg in 300 μl) may improve its efficacy as an antifungal lock solution. However, dissolving the higher concentrations of fluconazole into the small volumes needed for the antifungal lock technique proved to be difficult in this study.

Blood cultures drawn through the infected catheters in the antifungal lock experiments revealed some interesting observations. There seemed to be an exceptionally high rate of catheter thrombosis despite allowing heparin to dwell in the catheter lumens at all times. Thirty-three percent of catheters 3 days postinoculation were clotted. Of the cultures drawn at the completion of the treatment period, 57% of catheters were clotted in the untreated control, while only 14% of catheters were clotted in both the liposomal amphotericin B- and fluconazole-treated groups. The relationship between catheter thrombogenicity and catheter-related infection has been well documented in several studies (21–23, 27). However, it remains unclear whether catheter thrombosis promotes catheter-related infection or if the infection leads to thrombosis. In this study, more episodes of catheter thrombosis occurred in the untreated control group than the treated groups, suggesting that infection promotes catheter thrombosis.

Despite the complete sterilization of the catheters treated with liposomal amphotericin B by quantitative culture, 2 out of 7 blood cultures drawn from the catheters were positive for yeast. Although SEM of these catheters revealed almost completely clean intraluminal surfaces, each catheter examined had 1 to 2 small patches of *C. albicans* surrounded by minimal amounts of damaged matrix. There is a strong possibility that these adherent cells were dead, but their viability could not be

determined by our methods. These findings suggest that perhaps extending the treatment period to 10 to 14 days, as in the successful case reports with amphotericin B deoxycholate, may result in the complete eradication of fungal elements from catheters (14, 24).

Even though fluconazole lock therapy was unable to sterilize the intraluminal surface of catheters as documented by quantitative catheter culture and SEM, only 1 out of 7 catheter-drawn blood cultures grew yeast at the completion of the treatment period. The fluconazole lock solution may only kill free-floating planktonic *C. albicans* released from mature biofilm. The remaining yeast cells are encased in a thick extracellular matrix, as documented by SEM, which may not be released with routine blood draws through the catheter. Sonication of the treated catheters causes a disruption of the matrix with the release of yeast that is isolated by quantitative culture. Therefore, fluconazole lock therapy may represent an effective suppressive therapy for preventing continuous seeding of the bloodstream despite not sterilizing the catheter surface. A possible drawback to such a treatment strategy is the return of infection once the suppressive agent is stopped.

In summary, we describe the development of the first animal model of catheter-related *C. albicans* biofilm infection and show that this model has utility in preclinical evaluation of antifungal agents and in the study of biofilm pathogenesis. The demonstration of liposomal amphotericin B lock therapy as an effective strategy for treating *C. albicans* catheter-associated infections may have significant clinical implications, and a large prospective, randomized clinical trial should be performed in the near future.

ACKNOWLEDGMENTS

We thank A. Heuer and A. McIlwain at the Center for Surface Analysis of Materials for assistance with the SEM, A. Morrisey and the microbiology staff at University Hospitals of Cleveland for technical support with the blood cultures, T. J. Walsh for useful discussions during the development of the animal model, R. Rodriquez for help developing the surgical technique, D. Kuhn for critical review of the manuscript, and K. Smith for secretarial assistance.

Funding for this work was provided by the National Institutes of Health (Infectious Diseases/Geographic Medicine Training grant no. AI07024) to M.K.S. and (RO1 DE/AI 13932-01A1) to M.A.G., the American Heart Association (grant no. 0335313N) to P.K.M., the Dermatology Foundation to J.C., Fujisawa Healthcare to M.A.G., and Bristol-Myers Squibb (Award for Distinguished Research in Infectious Diseases) to M.A.G.

REFERENCES

1. **Anaissie, E. J., J. H. Rex, O. Uzun, and S. Vartivarian.** 1998. Predictors of adverse outcome in cancer patients with candidemia. Am. J. Med. **104:**238–245.
2. **Andris, D. A., E. A. Krzywda, C. E. Edmiston, C. J. Krepel, and C. M. Gohr.** 1998. Elimination of intraluminal colonization by antibiotic lock in silicone vascular catheters. Nutrition **14:**427–432.
3. **Bachmann, S. P., K. VandeWalle, G. Ramage, T. F. Patterson, B. L. Wickes, J. R. Graybill, and J. L. López-Ribot.** 2002. In vitro activity of caspofungin against *Candida albicans* biofilms. Antimicrob. Agents Chemother. **46:**3591–3596.
4. **Benoit, J. L., G. Carandang, M. Sitrin, and P. M. Arnow.** 1995. Intraluminal antibiotic treatment of central venous catheter infections in patients receiving parenteral nutrition at home. Clin. Infect. Dis. **21:**1286–1288.
5. **Carratala, J.** 2002. The antibiotic-lock technique for therapy of 'highly needed' infected catheters. Clin. Microbiol. Infect. **8:**282–289.
6. **Chandra, J., D. M. Kuhn, P. K. Mukherjee, L. L. Hoyer, T. McCormick, and M. A. Ghannoum.** 2001. Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. J. Bacteriol. **183:**5385–5394.
7. **Chandra, J., P. K. Mukherjee, S. D. Leidich, F. F. Faddoul, L. L. Hoyer, L. J. Douglas, and M. A. Ghannoum.** 2001. Antifungal resistance of candidal biofilms formed on denture acrylic *in vitro.* J. Dent. Res. **80:**903–908.
8. **Donlan, R. M.** 2001. Biofilm formation: a clinically relevant microbiological process. Clin. Infect. Dis. **33:**1387–1392.
9. **Edmond, M. B., S. E. Wallace, D. K. McClish, M. A. Pfaller, R. N. Jones, and R. P. Wenzel.** 1999. Nosocomial bloodstream infections in United States hospitals: a three-year analysis. Clin. Infect. Dis. **29:**239–244.
10. **Gudlaugsson, O., S. Gillespie, K. Lee, B. J. Vande, J. Hu, S. Messer, L. Herwaldt, M. Pfaller, and D. Diekema.** 2003. Attributable mortality of nosocomial candidemia, revisited. Clin. Infect. Dis. **37:**1172–1177.
11. **Hall, L. L., O. H. DeLopez, A. Roberts, and F. A. Smith.** 1974. Technical notes. A procedure for chronic intravenous catheterization in the rabbit. Lab. Anim. Sci. **24:**79–83.
12. **Hawser, S. P., and L. J. Douglas.** 1994. Biofilm formation by *Candida* species on the surface of catheter materials in vitro. Infect. Immun. **62:**915–921.
13. **Hawser, S. P., and L. J. Douglas.** 1995. Resistance of *Candida albicans* biofilms to antifungal agents in vitro. Antimicrob. Agents Chemother. **39:**2128–2131.
14. **Johnson, D. C., F. L. Johnson, and S. Goldman.** 1994. Preliminary results treating persistent central venous catheter infections with the antibiotic lock technique in pediatric patients. Pediatr. Infect. Dis. J. **13:**930–931.
15. **Kuhn, D. M., J. Chandra, P. K. Mukherjee, and M. A. Ghannoum.** 2002. Comparison of biofilms formed by *Candida albicans* and *Candida parapsilosis* on bioprosthetic surfaces. Infect. Immun. **70:**878–888.
16. **Kuhn, D. M., T. George, J. Chandra, P. K. Mukherjee, and M. A. Ghannoum.** 2002. Antifungal susceptibility of *Candida* biofilms: unique efficacy of amphotericin B lipid formulations and echinocandins. Antimicrob. Agents Chemother. **46:**1773–1780.
17. **Mermel, L. A., B. M. Farr, R. J. Sherertz, I. I. Raad, N. O'Grady, J. S. Harris, and D. E. Craven.** 2001. Guidelines for the management of intravascular catheter-related infections. Clin. Infect. Dis. **32:**1249–1272.
18. **Nucci, M., and E. Anaissie.** 2002. Should vascular catheters be removed from all patients with candidemia? An evidence-based review. Clin. Infect. Dis. **34:**591–599.
19. **O'Grady, N. P., M. Alexander, E. P. Dellinger, J. L. Gerberding, S. O. Heard, D. G. Maki, H. Masur, R. D. McCormick, L. A. Mermel, M. L. Pearson, I. I. Raad, A. Randolph, and R. A. Weinstein.** 2002. Guidelines for the prevention of intravascular catheter-related infections. Morb. Mortal. Wkly. Rep. Recomm. Rep. **51:**1–29.
20. **Raad, I., W. Costerton, U. Sabharwal, M. Sacilowski, E. Anaissie, and G. P. Bodey.** 1993. Ultrastructural analysis of indwelling vascular catheters: a quantitative relationship between luminal colonization and duration of placement. J. Infect. Dis. **168:**400–407.
21. **Raad, I. I., M. Luna, S. A. Khalil, J. W. Costerton, C. Lam, and G. P. Bodey.** 1994. The relationship between the thrombotic and infectious complications of central venous catheters. JAMA **271:**1014–1016.
22. **Stillman, R. M., F. Soliman, L. Garcia, and P. N. Sawyer.** 1977. Etiology of catheter-associated sepsis. Correlation with thrombogenicity. Arch. Surg. **112:**1497–1499.
23. **Timsit, J. F., J. C. Farkas, J. M. Boyer, J. B. Martin, B. Misset, B. Renaud, and J. Carlet.** 1998. Central vein catheter-related thrombosis in intensive care patients: incidence, risks factors, and relationship with catheter-related sepsis. Chest **114:**207–213.
24. **Viale, P., N. Petrosillo, L. Signorini, M. Puoti, and G. Carosi.** 2001. Should lock therapy always be avoided for central venous catheter-associated fungal bloodstream infections? Clin. Infect. Dis. **33:**1947–1948.
25. **Walsh, T. J., J. Bacher, and P. A. Pizzo.** 1988. Chronic silastic central venous catheterization for induction, maintenance and support of persistent granulocytopenia in rabbits. Lab. Anim. Sci. **38:**467–471.
26. **Wey, S. B., M. Mori, M. A. Pfaller, R. F. Woolson, and R. P. Wenzel.** 1988. Hospital-acquired candidemia. The attributable mortality and excess length of stay. Arch. Intern. Med. **148:**2642–2645.
27. **Yera, H., B. Sendid, N. Francois, D. Camus, and D. Poulain.** 2001. Contribution of serological tests and blood culture to the early diagnosis of systemic candidiasis. Eur. J. Clin. Microbiol. Infect. Dis. **20:**864–870.