Evaluation of Caspofungin and Amphotericin B Deoxycholate against Candida albicans Biofilms in an Experimental Intravascular Catheter Infection Model

Jennifer A. Shuford, Mark S. Rouse, Kerryl E. Piper, James M. Steckelberg, and Robin Patel

Division of Infectious Diseases, Department of Medicine, and Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, Minnesota

Candida albicans biofilms complicate the treatment of infected implanted intravascular devices because of decreased antifungal susceptibility. In our investigation, 48 rabbits with experimental central venous catheter C. albicans infection were equally allocated to a control arm or to receive amphotericin B deoxycholate or caspofungin treatment while undergoing systemic and intraluminal lock therapy for 7 days. C. albicans was cultured from catheters from all control rabbits, from 3 that received amphotericin B, and from 0 that received caspofungin. Differences in colony counts were detected between the control and amphotericin (P < .001) and control and caspofungin (P < .001) arms. Caspofungin may be useful in the treatment of C. albicans biofilm-associated intravascular catheter infections, which warrants further study.

Candidal biofilms play an important role in infections associated with medical devices. Conventional treatment of such infections includes the removal of the device in addition to the administration of antifungal agents [1]. Candida species are often associated with nosocomial bloodstream infections in patients with central venous catheters or other implanted devices [2, 3]; effective medical therapy of these biofilm-associated organisms that allows the retention of implanted devices would be a welcome advance.

Although initial in vitro studies of traditional antifungal agents against Candida albicans biofilms had disappointing results, more recent studies have demonstrated activity of lipid formulations of amphotericin and echinocandins against sessile C. albicans [4–6]. Despite these encouraging in vitro findings, to our knowledge, no published studies have described the activity of echinocandins in an animal model of C. albicans biofilm–associated infection. Therefore, we assessed the activity of caspofungin against C. albicans biofilm–associated central venous catheter infection in a rabbit model that reflects the pathogenesis of infection in humans.

Materials and methods. C. albicans IDRL-5319, a clinical strain isolated from blood culture, was approved for use by the Mayo Clinic Institutional Review Board. Mature biofilms were formed as described elsewhere [7, 8] for evaluation by scanning electron microscopy (SEM). Planktonic MIC values were determined using Clinical and Laboratory Standards Institute broth microdilution methods. C. albicans GDH2346, a well-characterized biofilm-producing strain, was used as a positive control. Sessile MIC values were determined using mature biofilms formed on microtiter plates, as described elsewhere [4, 9]; a reduction of absorption at 492 nm by 50% was set as the MIC.

Rabbit studies were developed and performed in accordance with the guidelines of the Institutional Animal Care and Use Committee at the Mayo Clinic (Rochester, MN). Male New Zealand White rabbits weighing 2.5–3.5 kg were used for all studies. The intravenous (iv) catheter implantation procedure was adapted from methods described elsewhere [10, 11]. Briefly, iv catheters were fashioned from silicone elastomer tubing (internal diameter, 0.04 inches; external diameter, 0.085 inches; Helix Medical) cut to a length of 31 cm and given a beveled tip. A 1-cm length of silicone elastomer tubing (internal diameter, 0.078 inches; external diameter, 0.125 inches; SF Medical) was slipped over the beveled tip of the catheter to form a cuff 4 cm from the tip. A Luer adaptor and Luer Lock injection hub (Qosina) were attached to the opposite end of the catheter. Catheters were sterilized using ethylene oxide.

Rabbits were anesthetized with ketamine (50 mg/kg intramuscularly [im]) and xylazine (3 mg/kg im). Their cervical and intrascapular regions were sheared with electric clippers, prepared with betadine solution, and draped in sterile fashion. The right external jugular vein was exposed through a 4–5-cm skin incision. Internal and external maxillary and right external jugular veins were dissected free of surrounding soft tissue. Up-
ward traction on the maxillary veins was maintained by a 4-0 silk suture (Ethicon) passed under the veins and clamped in a hemostat, which effectively reduced blood flow through the external jugular vein. A small incision was made in the external jugular vein using a Castroviejo scissor. The catheter was filled with sterile saline. The beveled tip of the catheter was inserted up to the cuff, which was then secured to the internal maxillary vein using a 4-0 prolene suture (Ethicon), leaving the external maxillary and external jugular veins patent. The cuff was sutured to the surrounding soft tissue for additional support. Fascia was closed over the catheter insertion site and the cuff. After the Luer adapter was removed, the opposite end of the catheter was clamped with a hemostat and tunneled between fascial planes from the insertion site to an exit site in the prepared intrascapular region. The catheter was cut to length, and the Luer adaptor and hub were replaced on the tubing. The adaptor was buried subcutaneously. The hub remained exposed above the skin surface. Anchor sutures were placed on either side of the hub and looped several times around the hub; then a knot was tied.

Next, 15 mg/kg of vancomycin (Novaplus; Abbot Laboratories) was immediately administered through the catheter as prophylaxis as an iv slow infusion; this was followed by the administration of heparinized saline. Then, 0.1 mg of buprinorphine was administered im for analgesia. One day later, cfu of $7 \times 10^7$ C. albicans, IDRL-5319 in 1 mL of sterile saline was infused through the catheter over the course of 2 min. After the infusion of C. albicans, and then daily for 2 more days, the catheter was flushed and locked with 100 U of heparin in 300 mL of sterile saline. Three days after infection, when aspiration was possible, blood was drawn through the catheters for culture.

Preliminary studies were performed with 6 rabbits whose catheters underwent daily flushing for 5 days with 300 $\mu$L of sterile saline that contained 100 U of heparin. Four catheters were cultured, as described below; all tested positive for Candida infection. The remaining 2 catheters were evaluated by SEM; candidal biofilms were visualized on internal and external catheter surfaces in both cases.

Three days after infection, treatment was initiated. Sixteen rabbits were allocated to each of 3 study arms—caspofungin, amphotericin B deoxycholate, or control. The control arm underwent daily catheter flushes with 100 U of heparin in 300 $\mu$L of sterile saline during the treatment period.

Caspofungin was purchased from the Mayo Clinic pharmacy (Cancidas; Merck), reconstituted in sterile saline, and used within 24 h. Once daily, 1 mg/kg of caspofungin was infused through the catheter over the course of 1 min. After infusion, a 300-$\mu$L solution that contained 100 U of heparin and 2 mg of caspofungin in sterile saline was instilled into the catheter as lock therapy. Amphotericin B deoxycholate (X-Gen Pharmaceuticals) was purchased from the Mayo Clinic pharmacy, reconstituted in sterile water, and used within 24 h. Once daily, 1 mg/kg of amphotericin was diluted in D$_5$W (Baxter) and infused through the catheter at a rate of 0.1 mg every 15 s. After the infusion of amphotericin, a 300-$\mu$L solution that contained 100 U of heparin and 1 mg of amphotericin in sterile water was instilled into the catheter as lock therapy. In each study arm, treatment was administered for 7 days.

Ten days after infection and 24 h after the last treatment, rabbits were anesthetized with 50 mg/kg each of ketamine and xylazine, both im. Blood was collected for culture via cardiac puncture and through the catheter, when blood aspiration was possible. Rabbits were euthanized via the intracardiac infusion of 100 mg/kg of pentobarbital. Each catheter was dissected from surrounding tissue using sterile technique, was bisected lengthwise, and was placed in 5 mL of sterile saline. Catheters were vortexed for 30 s, followed by sonication (at 40 kHz and 320 mW/cm$^2$ in a table-top ultrasonic cleaner system, generator model G2-80/40MT and tank model T400-1; Zenith Ultrasonics) for 1 min. This was repeated for a total of 3 cycles, followed by 30 s of vortexing. Suspensions were poured through sterile 0.22-$\mu$m filters and rinsed with 250 mL of sterile saline for the removal of residual antimicrobial agents from harvested organisms. Filters were segmented and placed in 5 mL of sterile saline before undergoing 3 additional cycles of vortexing and sonication, as described above. Serial dilutions were made, and 100-$\mu$L aliquots were plated on Sabouraud dextrose agar (Remel) and blood agar (Becton Dickinson). For blood culture, 100-$\mu$L samples were plated on Sabouraud dextrose agar and incubated for 48 h at 37°C before colonies were counted.

Differences in mean log$_{10}$ colony-forming units per catheter were compared with the control arm (**). No significant difference was found between the treatment arms (**P = .36).
between treatment arms were evaluated using the Wilcoxon rank sum test. \( P < .05 \) was considered to be statistically significant.

**Results.** *C. albicans* IDRL-5319 planktonic MIC values for amphotericin and caspofungin were 0.25 and 0.5 \( \mu \)g/mL, respectively. Biofilm MIC values for both amphotericin and caspofungin were 0.5 \( \mu \)g/mL. The ability of *C. albicans* IDRL-5319 to form a biofilm on silicone elastomer was verified by SEM.

Results of treatment are shown in figure 1. *C. albicans* was cultured from all harvested catheters in the control group at a mean \( \pm \) SD of 4.72 \( \pm \) 0.94 log\(_{10}\) cfu. For rabbits treated with amphotericin, 13 of 16 catheters were sterile, with a mean \( \pm \) SD of 0.68 \( \pm \) 1.53 log\(_{10}\) cfu. All catheters from caspofungin-treated rabbits were sterile. Differences in colony counts were significant when catheters from control rabbits were compared with those from amphotericin-treated \( (P < .001) \) or caspofungin-treated \( (P < .001) \) rabbits. There was no statistically significant difference between colony counts of catheters from amphotericin- and caspofungin-treated rabbits \( (P = .36) \).

At the time of pretreatment blood culture, 12, 13, and 15 catheters from the control, amphotericin-treated, and caspofungin-treated rabbits, respectively, were clotted, which interfered with the aspiration of blood. All blood cultures drawn through patent catheters before treatment yielded *C. albicans*. No blood cultures were obtained through the catheters after the treatment period because of clotting, although all catheters continued to function well for infusion. Two posttreatment blood cultures, obtained by cardiac puncture before rabbits were killed, tested positive for *C. albicans* in the control group, whereas all posttreatment blood cultures were negative in the amphotericin- and caspofungin-treated groups.

**Discussion.** We demonstrated that the combination of systemic and intraluminal lock therapy with either caspofungin or amphotericin B deoxycholate significantly reduced colony counts of *C. albicans* associated with catheters, resulting in sterile catheters in many cases. Specifically, all catheters in the caspofungin-treated group were sterile when evaluated by quantitative culture after 7 days of intravenous and intraluminal therapy.

Promising results of antifungal lock therapy were published in 2004 by Schinabeck et al. [11] that described significant decreases in *C. albicans* colony-forming units in catheter-related infections when fluconazole or liposomal amphotericin intraluminal therapy was used. However, their study design differed from ours and may have been less clinically relevant. In their study, intravascular catheter infections were established and visualized only within the catheter lumens. Subsequent treatment was likewise limited to the catheter lumens. Also, their animal model lacked blood flow around the outer surface of the intravascular portion of the catheter, because the external jugular vein was ligated near the insertion site.

Our design likely reflects more closely human intravascular catheter infection, because blood flow was maintained through the external jugular vein (i.e., via the external maxillary vein) after catheter placement. Also, our preliminary studies demonstrated biofilm on both intra- and extraluminal surfaces, as occurs in human catheter infections [12].

We included both systemic and intraluminal therapy in our study design, because biofilm on the extraluminal surface is not addressed by intraluminal therapy. Previous in vitro work has demonstrated the superior activity of echinocandins, compared with traditional antifungal agents, in the treatment of *C. albicans* biofilms [4–6]; MIC values of echinocandins have been reported that fell within the range of clinically achievable serum concentrations [5]. Because echinocandins are generally well tolerated, they deserve further study in the treatment of biofilm-related fungal diseases.

Amphotericin therapy demonstrated greater efficacy in our study than we expected. Lock therapy results in high intraluminal concentrations of amphotericin that exceed the MIC of the biofilm-associated organisms within the lumen of the catheter, but the serum concentration of amphotericin is not usually high enough to exceed the MIC of the biofilm-associated organisms on the extraluminal surface. The unexpected outcomes in our study may have resulted from a higher concentration of amphotericin near the surface of the catheter than the average serum concentration, from the assistance of an active immune system, or from characteristics of the study strain. Although the difference in culture results between amphotericin- and caspofungin-treated catheters did not reach statistical significance, this may have been due to the small sample sizes.

Our study had several limitations. *C. albicans* organisms were slowly infused through the catheter in suspension. Although this differs from the pathogenesis of some human infections, colonization of both surfaces of the catheter was documented, which is similar to what happens in human infection. Clotting in the catheters precluded blood aspiration from every catheter before treatment. However, every blood sample obtained through the pretreatment catheters and every harvested control catheter grew *C. albicans* when cultured. Therefore, our method of infecting sterile catheters appears to be reliable. Finally, all studies were performed with one strain of *C. albicans*. Our results may or may not be applicable to other strains of *C. albicans*.

**Acknowledgments**

We thank Jennifer Milverstad and Rachel C. Steckelberg, for assistance with the in vitro studies; Melissa J. Jacobson, for assistance with the in vivo study; and Jyotsna Chandra, Mahmoud A. Ghannoum, and L. Julia Douglas, for use of the GDH2346 isolate.

**References**


