

## COLONIZATION-RESISTANT ANTIMICROBIAL-COATED PERITONEAL DIALYSIS CATHETERS: EVALUATION IN A NEWLY DEVELOPED RAT MODEL OF PERSISTENT *PSEUDOMONAS AERUGINOSA* PERITONITIS

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◆ **Objective:** Development of a rat model of persistent peritonitis and evaluation of the ability of liposomal ciprofloxacin hydrogel-coated silicone to resist colonization.

◆ **Design:** A newly developed model of persistent *Pseudomonas aeruginosa* peritonitis to compare the ability of liposomal ciprofloxacin hydrogel (LCH)-coated silicone versus plain silicone for resistance to bacterial colonization.

◆ **Animals:** Male Sprague-Dawley rats.

◆ **Results:** Inoculating the peritoneum of rats with 1 mL 0.5% agar containing  $10^6$  colony-forming units (cfu)/mL *P. aeruginosa* in the presence of a plain silicone coupon resulted in persistent peritonitis for at least 7 days. Plain silicone coupons in all 40 rats were colonized (median  $2.54 \times 10^3$  cfu/cm<sup>2</sup>; range  $5.0 \times 10^1 - 1.0 \times 10^6$  cfu/cm<sup>2</sup>) and peritoneal washings were consistently culture-positive. In contrast, the LCH coupons removed after 7 days from the 40 test rats were sterile, as were the peritoneal washings, and there was no evidence of peritonitis. Blood cultures were negative in both groups.

◆ **Conclusions:** Liposomal ciprofloxacin hydrogel-coated silicone resists colonization in this rat model of persistent *P. aeruginosa* peritonitis.

**KEY WORDS:** Persistent peritonitis; antimicrobial coating; ciprofloxacin; *Pseudomonas aeruginosa*; rats.

A major complication of continuous ambulatory peritoneal dialysis (CAPD) is infection. The incidence of peritonitis varies, but a rate of 1 – 1.3 patient-episodes per year has been reported (1). Most infections (40% – 60%) are caused by coagulase-negative staphylococci, with an additional 10% each being due to *Staphylococcus aureus* and *Pseudomonas aeruginosa* (2). However, *S. aureus*, *P. aeruginosa*, and *Candida spp* are responsible for the majority of CAPD infections that necessitate catheter removal (2). The

recommended course of action for the treatment of persistent peritonitis resistant to antibiotics is to remove the catheter, support the patient with hemodialysis for several weeks during antibiotic therapy to resolve the peritonitis, and then insert a new peritoneal dialysis (PD) catheter (3). Studies have shown that PD catheter-related infections and/or peritonitis are responsible for 30% of permanent patient transfers to hemodialysis (4).

Under certain circumstances, peritonitis has been treated successfully by removal and immediate replacement of the PD catheter, with concomitant antibiotic therapy (5,6). Advantages of simultaneous exchange of infected catheters include the maintenance of dialysis through continued PD, reduction of the transfer rate to hemodialysis, and elimination of a second operative procedure. The overall success rate of simultaneous PD catheter exchange ranges from 65% – 98% (7). With simultaneous exchange for exit-site infections and recurrent peritonitis, success rates range from 87% – 94%, compared to 58% in cases of persistent peritonitis caused by organisms recalcitrant to treatment (7). Placing a new catheter into an infected peritoneum will result in prompt colonization of the replacement catheter. In peritonitis secondary to *S. aureus*, *P. aeruginosa*, *Mycobacterium spp*, fecal organisms, and *Candida spp*, the current recommendation is sequential exchange, with insertion of the new catheter only after the infectious and inflammatory processes have resolved (5).

Persistent PD-related infections are the result of bacterial adhesion to biomaterial surfaces and the subsequent formation of antibiotic-resistant biofilms that are difficult to treat *in situ*. There have been attempts to develop biomedical products that resist bacterial colonization and reduce infection in other clinical settings. We have shown that liposomal ciprofloxacin gelatin-polyethylene glycol hydrogel (LCH)-coated Foley catheters resist colonization by uropathogens in a rabbit model for up to 7 days (8).

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However, the safety and efficacy of placing this biomaterial in an infected peritoneum are unknown. Thus, development of an animal model of persistent PD catheter-related peritonitis was necessary. Most published models of PD peritonitis involve the introduction of previously contaminated catheter materials or contaminated dialysate into the peritoneum prior to therapy, or artificial contamination of the catheter exit site (9–12). To our knowledge, there are no published models for testing antimicrobial PD catheter materials in the presence of peritonitis.

The purpose of this study was to develop a rat model of persistent *P. aeruginosa* peritonitis, without bacteremia, and to evaluate the efficacy of LCH-coated silicone as a potential biomaterial for PD catheters to be used in simultaneous exchange surgery for peritonitis.

## MATERIALS AND METHODS

### ANIMALS

Male Sprague–Dawley rats weighing 200–250 g (Charles River) were used throughout the study. They were kept in regular housing, 2 per cage, until infected, then housed in containment. The animals were fed and watered *ad libitum* as per Canadian Council on Animal Care guidelines. They were inspected daily for signs of illness or sepsis and immediately euthanized if found to be in distress.

### MICRO-ORGANISMS

A ciprofloxacin-sensitive clinical isolate of *P. aeruginosa* obtained from a patient who had developed persistent PD catheter-related peritonitis was used for all experiments. The strain was maintained at –70°C in Luria–Bertani medium (LB, Difco; Becton Dickinson, Franklin Lakes, New Jersey, USA) in 10% glycerol. Working preparations were made by inoculating 50 mL of nutrient broth with the frozen stock and growing for 18 hours at 37°C with agitation. The bacteria were harvested by centrifugation, washed in sterile isotonic saline, and resuspended to  $2 \times 10^6$  colony-forming units (cfu)/mL in sterile saline. This suspension (0.5 mL) was then added to 0.5 mL sterile 1% agar to produce 1 mL of 0.5% agar containing  $10^6$  bacteria, which served as the inoculum to infect the peritoneum.

### SILICONE COUPONS

Sterile silicone coupons (1 × 2 × 0.2 cm; Pillar Surgical, La Jolla, California, USA) were used as a model of PD catheters. Encapsulation of ciprofloxacin, synthesis of liposomal hydrogel, and coating of silicone

coupons with LCH were carried out as previously described (13). Briefly, sterile silicone coupons were coated with liposomal hydrogel-encapsulated ciprofloxacin under sterile conditions, and the coated coupons were surface-sterilized with ultraviolet light. Coupons from each batch were randomly tested for sterility.

### EXPERIMENTAL MODEL

The rats were anesthetized by halothane inhalation with nitrous oxide. The proposed incision site was cleansed with antiseptic solution and a 2-cm midline laparotomy incision was made. Omentectomy and excision of gubernacular tissue surrounding the spermatic cord was performed, as preliminary biocompatibility studies showed that these tissues would envelop the coupon, potentially altering the dynamics of antibiotic release or bacterial colonization. The silicone coupon was placed into the right lower quadrant of the peritoneal cavity. Forty rats each received either a plain silicone coupon or an LCH coupon. Agar (1 mL, 0.5%) containing  $10^6$  *P. aeruginosa* was distributed throughout the peritoneal cavity, including on the coupon, with a sterile transfer pipette. The incision was closed in two layers.

After 7 days, the rats were sacrificed by CO<sub>2</sub>. A sternotomy incision was made and blood was drawn from the heart with a sterile 1-mL syringe fitted with a 23-gauge needle. Blood (500 µL) drawn from the heart was plated on sheep's blood agar to identify bacteremic animals. A midline laparotomy incision through the initial wound site was made and the peritoneum and viscera were examined for signs of infection. The coupons were retrieved and measurements of viability of bacteria adherent to the coupons were determined as previously described (14). Briefly, the coupon was rinsed in sterile saline to remove nonadherent material/bacteria, then placed in 5 mL sterile phosphate-buffered saline (PBS) containing five or six 3-mm diameter glass beads. The coupons were subjected to three 30-second rounds of sonication on ice followed by vortexing for 30 seconds to dislodge biofilm bacteria. The suspension was serially diluted and plated on nutrient agar for overnight growth at 37°C. The presence of bacteria in the peritoneum was determined by tenfold serial dilution and plate counts of 1 mL PBS recovered after irrigation of the peritoneal walls and cavity.

### STATISTICAL ANALYSIS

Bacterial plate counts were recorded and the median number of cfu per unit area of the original coupon was determined. The nonparametric Mann–

Whitney U test was used to compare rates of coupon colonization.

## RESULTS

### MODEL DEVELOPMENT

Initial attempts to use a fibrin clot-based rat model, previously used to investigate intra-abdominal abscess-associated sepsis (15), were not successful. The fibrin clots contracted and extruded bacterial suspension in an inconsistent fashion. Thus, it was not possible to reproducibly generate a fixed quantity of embedded bacteria to act as an inoculum. An "agar bead" model previously developed to study chronic pulmonary infections in cystic fibrosis (16) was adapted as the basis of our model. Instead of entrapping bacteria in agar beads, semisolid 0.5% agar was used. The material was broken up with a sterile transfer pipette and distributed about the peritoneal cavity. Based on the fibrin clot data (12), an initial inoculum of  $10^8$  cfu/mL was used. However, this dose of *P. aeruginosa* resulted in sepsis and death within 48 hours, thus the dose was reduced two logs to  $10^6$ . Instillation of 1 mL 0.5% agar containing  $10^6$  bacteria into the peritoneum resulted in diffuse peritonitis. The finding of culture-positive peritoneal washings at 7 days provided further evidence of persistent peritonitis.

During preliminary biocompatibility experiments, it was noted that the omentum and/or gubernaculum would adhere to and cover the hydrogel coupons, potentially impeding bacterial colonization of the coupon. Based on this concern, omentectomies and bilateral excision of the gubernaculum was performed on all animals. No other adverse effects were noted.

### COMPARISON OF LCH COUPONS TO PLAIN SILICONE COUPONS

The animal model was then used to test the ability of LCH-coated coupons to resist bacterial colonization compared with uncoated coupons. Comparison of the control rats to those receiving LCH coupons showed a marked difference in the appearance of the peritoneum. Although the rats in both groups appeared grossly normal prior to autopsy, the peritoneum and viscera of the control animals (plain silicone) had the typical signs of chronic peritonitis. Exudate and bowel adhesions with occasional walled-off abscesses were present (Figure 1). Also, the bowels of the controls were dilated, possibly reflecting an ileus. Their incisions were healing slowly and the majority appeared infected on the undersurface. In contrast, those implanted with LCH coupons showed no signs of peritonitis. Their wounds healed normally without evidence of infection (Figure 1).

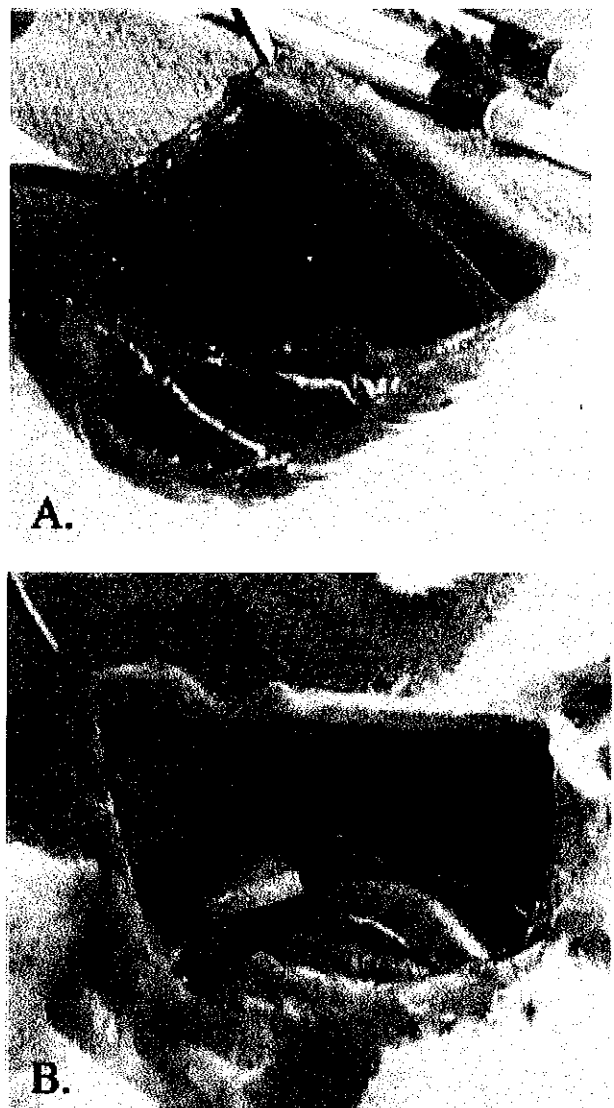


Figure 1 — Postmortem images of the peritoneum from a rat that received a plain silicone coupon (A) and one that received a liposomal ciprofloxacin hydrogel (LCH)-coated coupon (B). Note bowel adhesion and gross peritoneal inflammation in A compared to the LCH coupon lying freely in the normal appearing peritoneal cavity in B.

Cultures of heart blood from both groups failed to grow bacteria, demonstrating that peritonitis in this model was not associated with bacteremia. Cultures derived from sonication and vortexing of the coupons grew *P. aeruginosa* in all animals that had received plain silicone coupons. The median number of bacteria was  $1.27 \times 10^4$  cfu/mL (range  $2.5 \times 10^2$  to  $5 \times 10^6$  cfu/mL) or  $2.54 \times 10^3$  cfu/cm<sup>2</sup> (range  $5.0 \times 10$  to  $1.0 \times 10^6$  cfu/cm<sup>2</sup>). The LCH coupons were consistently culture-negative. With no colonization in the LCH silicone test group, the difference was obviously statistically significant ( $p < 0.01$ ). Cultures of random

peritoneal washings from the LCH silicone group were also negative. However, peritoneal washings of five randomly chosen animals from the control group were culture-positive, with a median of  $5.8 \times 10^3$  cfu/mL (range  $1.7 \times 10^3$  to  $3.5 \times 10^4$  cfu/mL).

## DISCUSSION

Peritonitis is a major complication of CAPD and is responsible for a number of permanent patient transfers to hemodialysis. Recently, peritonitis has been treated with varied success by removal and immediate replacement of the PD catheter at a new site, with concomitant antibiotic therapy. Advantages of simultaneous exchange of infected catheters include the maintenance of dialysis capacity through continued PD, reduction of the transfer rate to hemodialysis, and elimination of a second operative procedure. However, placing a new catheter into an infected peritoneum results in prompt colonization of the replacement catheter and subsequent risk of recurrence. Our ultimate goal is to develop a PD catheter coating that resists colonization when introduced into an infected peritoneum.

In order to develop and test colonization-resistant PD catheter coatings, development of an animal model was necessary. Peritoneal dialysis catheter-related peritonitis is rarely associated with positive blood cultures (17). Introduction of 1 mL 0.5% agar containing  $10^6$  cfu/mL *P. aeruginosa* into the peritoneum of rats resulted in persistent peritonitis with consistently culture-positive peritoneal washings at 7 days, without bacteremia.

Despite bacteriological challenge, all the LCH-coated coupons resisted colonization for the study period. Rats receiving the coated coupons were free of peritonitis and furthermore, had well-healed incisions. LCH was initially chosen for this study because of our experience with this biomaterial as a Foley catheter coating (13). Previous *in vitro* studies showed that these surfaces release ciprofloxacin on a continuous basis at approximately  $2.0 \mu\text{g}/\text{cm}^2/\text{hour}$  for several days (13). Regarding ciprofloxacin release, it is possible that a small amount of antibiotic is also liberated from the coupon during sonication with glass beads and thus becomes part of the bacterial suspension that is plated on nutrient agar. However, released cells are immediately subjected to serial dilution in large volumes of sterile PBS that would reduce the concentration to below the minimum inhibitory concentration. A small volume of bacterial suspension is then placed on a nonselective agar surface, further reducing the local antibiotic concentration by diffusion. Therefore, contact with any released antibiotic is brief and unlikely to cause killing. Peritoneal washings from animals with

LCH coupons were plated directly without further manipulations and were consistently negative, suggesting that no live bacteria were present at the time of necropsy.

This study confirmed the efficacy of this antibiotic coating in resisting colonization when exposed to *P. aeruginosa* PD peritonitis; however, *S. aureus* is also a common cause of persistent PD peritonitis. *Staphylococcus aureus* is less sensitive to ciprofloxacin and thus an alternate antimicrobial would likely be more effective. The liposomal hydrogel system we employed allows for encapsulation of other antimicrobials. Ultimately, we envision this system enabling the use of antimicrobial-specific coatings for individualized treatment of infections.

In summary, we have developed an animal model of chronic peritonitis and shown the efficacy of ciprofloxacin hydrogel-coated silicone to resist colonization when exposed to *P. aeruginosa* peritonitis. Furthermore, our model will enable us to study newer biomaterials that may ultimately allow for simultaneous exchange with an antimicrobial PD catheter for patients suffering from persistent PD catheter-related peritonitis.

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