Fibrinogen Release and Deposition on Urinary Catheters Placed during Urological Procedures

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Abbreviations and Acronyms

CAUTI = catheter associated UTI

PBS = phosphate buffered saline

PBS-T = PBS containing 0.05% Tween® 20

UTI = urinary tract infection

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Purpose: Catheter associated urinary tract infections account for approximately 40% of all hospital acquired infections worldwide with more than 1 million cases diagnosed annually. Recent data from a catheter associated urinary tract infection animal model has shown that inflammation induced by catheterization releases host fibrinogen, which accumulates on the catheter. Further, *Enterococcus faecalis* catheter colonization was found to depend on EbpA (endocarditis and biofilm-associated pilus), a fibrinogen binding adhesin. We evaluated this mechanism in a human model.

Materials and Methods: Urinary catheters were collected from patients hospitalized for surgical or nonsurgical urological procedures. Catheters were subjected to immunofluorescence analyses by incubation with antifibrinogen antibody and then staining for fluorescence. Fluorescence intensity was compared to that of standard catheters. Catheters were incubated with strains of *Enterococcus faecalis, Staphylococcus aureus* or *Candida* to assess binding of those strains to fibrinogen laden catheters.

Results: After various surgical and urological procedures, 50 catheters were collected. *In vivo* dwell time ranged from 1 hour to 59 days. All catheters had fibrinogen deposition. Accumulation depended on dwell time but not on surgical procedure or catheter material. Catheters were probed *ex vivo* with *E. faecalis*, *S. aureus* and *Candida albicans,* which bound to catheters only in regions where fibrinogen was deposited.

Conclusions: Taken together, these data show that urinary catheters act as a binding surface for the accumulation of fibrinogen. Fibrinogen is released due to inflammation resulting from a urological procedure or catheter placement, creating a niche that can be exploited by uropathogens to cause catheter associated urinary tract infections.

Key Words: urinary tract infections, iatrogenic disease, catheterization, fibrinogen, hospitalization

Urinary tract infections represent a significant burden to the health care system, affecting approximately 150 million people worldwide[1,2] with an estimated annual cost of $2 to $3 billion in the United States.[2] Serious sequelae can result from UTIs, including pyelonephritis and

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bacteremia, causing severe morbidity and mortality.[3] Hospitalization is a risk factor for UTI with an estimated risk on any day of 1.1% to 6.5%. Catheterized patients are at substantially higher risk than patients without exposure to a catheter,[4] and bacteriuria develops within 30 days of catheter placement in virtually all patients.[1,5,6]

Urinary catheters are the second leading cause of hospital acquired bacteremia, leading to a 7-day mortality rate greater than 30%.[7] Furthermore, the emergence of multidrug resistant microorganisms such as VRE (vancomycin resistant enterococci) and MRSA (methicillin resistant *Staphylococcus aureus*), and fluoroquinolone resistance in *Escherichia coli* is of critical concern.[2,8,9] The overuse of antibiotics during UTIs can lead to fungal infection and this is a universal risk for acquiring a *Candida* UTI.[10] Therefore, understanding the interplay between uropathogen virulence factors and the patient response will pave the way for the development of alternative treatments to reduce antibiotic use.

In a murine model[11] fibrinogen deposition on catheters is the key pathogenic event leading to colonization by *Enterococcus faecalis*.[12] In this model circulating fibrinogen increases in response to tissue damage from catheter placement, enters the bladder and is deposited on catheters in a time dependent manner. *E. faecalis* adheres to the deposited fibrinogen using Ebp, a hair-like heteropolymeric surface protein.[12–14] The EbpA subunit located at the tip of the fiber binds fibrinogen to facilitate catheter associated biofilm formation.[12,15] Vaccination with EbpA attenuates *E. faecalis* colonization and biofilm formation during catheterization.[12]

The goal of this study was to translate these observations from the murine model to human disease by 1) investigating the deposition of fibrinogen on human urinary catheters and 2) using an *ex vivo* assay to assess whether fibrinogen deposits can potentiate colonization by several common uropathogens, including *E. faecalis*, *S. aureus* and *Candida albicans*.

METHODS

Bacterial Strains and Growth Conditions

Strains included *E. faecalis* OG1RF (SJH-1994), an isogenic EbpA mutant that can express Ebp but cannot bind to fibrinogen (SJH-2001),[12] as well as *S. aureus* (SJH-1369) and *C. albicans* (SJH-3140). For liquid cultures Brain Heart Infusion Broth (BD®) was used, which was inoculated from a single colony grown on Brain Heart Infusion plates. Cultures were grown at 37C for 18 hours while shaking at 200 rpm for *S. aureus* and *C. albicans*, and statically for *E. faecalis*.

Antibodies

The primary antibodies were goat anti-human fibrinogen (No. F8512), rabbit anti-Protein A antigen (anti-*S. aureus*) (No. P3775; Sigma-Aldrich®), rabbit anti-Streptococcus group D antigen (anti-*E. faecalis*) (Lee Laboratories, Grayson, Georgia)[12] and rabbit anti-*C. albicans* (No. ab53891, Abcam®). The secondary antibodies were IRDye® 800CW donkey anti-goat (No. 926-32213); and IRDye 680LT goat anti-rabbit (No. 926-68021).

Urinary Catheter Collection

Urinary catheters consisting of 100% silicone, silicone elastomer or latex were placed for surgical or nonsurgical urological procedures in consented patients (supplementary table, http://jurology.com/). The gender, procedure type, catheter material, dwell time, prior urine culture and antibiotics prescribed were recorded for each patient. This study was approved by the Washington University School of Medicine internal review board (approval No. 201410058).

Fibrinogen

Deposition Analysis. After collection the catheters were cut into 10 cm segments, fixed with formalin for 1 hour and washed 3 times with PBS. For analysis, the first 10 cm of the catheter tip was blocked by overnight incubation at 4C with 1.5% bovine serum albumin with 0.1 % sodium azide in PBS. The tip was then washed 3 times for 5 minutes each with PBS-T. A 15 ml solution containing goat anti-human fibrinogen diluted 1:500 in PBS-T was added and incubated at room temperature for 2 hours. Catheters were then washed 3 times for 5 minutes each with PBS-T and incubated with donkey anti-goat IRDye 800CW (diluted 1:10,000) for 45 minutes at room temperature. Following an additional 3 washes with PBS-T the catheters were examined for an infrared signal using the Odyssey® Imaging System. Autofluorescence was assessed in nonimplanted catheters and in catheters not incubated with the primary antibody, which was minimal.

Quantification. The concentration of fibrinogen deposited on clinical catheters was determined by comparing the infrared signal following staining to that of a standard curve prepared by incubating fibrinogen (0.0001 to 10 mg/ml) with nonimplanted catheters that were then processed as described. Separate curves were generated for each type of catheter material. A linear relationship model was used to compare the amount of fibrinogen deposition to variables (dwell time, surgical procedure and bacterial positive urine cultures) that were tested for significance by the Pearson correlation using Prism®.

Ex Vivo Binding to Catheters

Catheters from patients with negative urine cultures were treated as described except antibody was replaced with uropathogens. Overnight cultures were washed and resuspended in PBS. Cell density was normalized to an OD_{600} (optical density measured at 600 nm) of 0.5, including approximately 2×10^7 cfu for *E. faecalis* and *S. aureus* strains, and approximately 2×10^4 cfu for *C. albicans*. The suspension (15 ml) was incubated with the patient catheter at 37C with shaking conditions (100 rpm). Uninfected control catheters were incubated with PBS alone. At 1 hour catheters were washed 3 times with