The Role of Staphylothrombin-Mediated Fibrin Deposition in Catheter-Related \textit{Staphylococcus aureus} Infections

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\textit{Staphylococcus aureus} (\textit{S. aureus}) is a frequent cause of catheter-related infections. \textit{S. aureus} secretes the coagulas staphylocoagulase and von Willebrand factor–binding protein, both of which form a staphylothrombin complex upon binding to prothrombin. Although fibrinogen and fibrin facilitate the adhesion of \textit{S. aureus} to catheters, the contribution of staphylothrombin-mediated fibrin has not been examined. In this study, we use a \textit{S. aureus} mutant lacking both coagulases (\textit{Δcoa/vwb}) and dabigatran, a pharmacological inhibitor of both staphylothrombin and thrombin, to address this question. Genetic absence or chemical inhibition of pathogen-driven coagulation reduced both fibrin deposition and the retention of \textit{S. aureus} on catheters in vitro. In a mouse model of jugular vein catheter infection, dabigatran reduced bacterial load on jugular vein catheters, as well as metastatic kidney infection. Importantly, inhibition of staphylothrombin improved the efficacy of vancomycin treatment both in vitro and in the mouse model.

\textbf{Keywords.} \textit{Staphylococcus aureus}; staphylothrombin; staphylocoagulase; von Willebrand factor-binding protein; dabigatran; catheter infection.

The use of intravascular catheters is essential in medical practice. However, by providing a vascular access for the administration of intravenous therapy, intravascular catheters also constitute a breach in the barrier between the outside milieu and the bloodstream, thus increasing the risk of intravascular infections. Although efforts to reduce catheter-related bloodstream infections (CRBSIs) are increasing, nosocomial infections associated with central venous catheters remain a significant problem. The incidence of CRBSI in the United States is approximately 80,000 cases/year in intensive care units alone and up to 250,000 cases/year in total [1], with an attributable mortality of up to 35% in some series [2, 3]. With an additional cost of about US$35,000 per episode, CRBSIs increase healthcare expenditure by several billion dollars per year [4].

\textit{Staphylococcus aureus} is one of the most frequent causes of CRBSIs, accounting for 15%–30% of all cases in the general population [5]. In addition, patients undergoing chronic renal replacement therapy [6], geriatric patients [7], and cancer patients seem to be particularly at risk for \textit{S. aureus} CRBSI [8]. Furthermore, CRBSIs due to \textit{S. aureus} are also associated with a higher incidence of severe sepsis and metastatic infectious complications, such as infective endocarditis, osteomyelitis, and abscesses, as well as with treatment failure and recurrent infections [6, 9].

The ability of pathogens to adhere to and persist on the surface of intravascular devices is crucial to the pathogenesis of CRBSIs. Once catheters are inserted in the bloodstream, they are rapidly coated with circulating
plasma proteins, such as fibrinogen [10]. *S. aureus* expresses a range of surface receptors, the microbial surface components recognizing adhesive matrix molecules, which bind to these host proteins and mediate the initial step in bacterial adhesion [11, 12]. In a second step, a stable protective 3-dimensional structure of adherent biomaterial increases the attachment of bacteria to catheter surfaces and shields the pathogen from the immune system. Furthermore, embedding of pathogens in an extracellular matrix may reduce the sensitivity to antibiotics [13].

One of the hallmarks of *S. aureus* is the ability to activate the human coagulation system by secreting 2 coagulases, staphylocoagulase [14] and von Willebrand factor–binding protein [15, 16], both of which bind to prothrombin to form a 1:1 complex referred to as staphylothrombin. The association of either staphylocoagulase or von Willebrand factor–binding protein with prothrombin to form staphylothrombin induces a conformational change resulting in the exposure of the active site of thrombin, which converts soluble fibrinogen into insoluble fibrin strands. This conformational activation of prothrombin to staphylothrombin bypasses the physiological control of the coagulation cascade and renders staphylothrombin insensitive to inactivation by antithrombin and anticoagulant drugs, such as (low-molecular-weight) heparins (LMWHs). In contrast, staphylothrombin can be inhibited by the direct thrombin inhibitors argatroban [17] and dabigatran [18].

We have previously shown that fibrin deposition by *S. aureus* reduced the activation of polymorphonuclear leukocytes, thus increasing *S. aureus* resistance to phagocytes [19]. Furthermore, staphylothrombin activity increased the capacity to cause metastatic infectious foci in a murine model of *S. aureus* bacteremia [20, 21] and increased the retention of *S. aureus* to foreign surfaces in vitro [19].

We aimed to study the role of this pathogen-driven fibrin deposition by staphylothrombin in the retention and persistence of *S. aureus* on catheters and the potential of staphylothrombin inhibition as an adjunctive strategy in the prevention or treatment of *S. aureus* catheter-related infections.

**METHODS**

**Bacterial Strains**

*S. aureus* Newman used in this study was originally isolated from a case of osteomyelitis secondary to tuberculosis [22]. The isogenic *S. aureus* Newman Δcoa/vwb variant has been previously described [21]. This double mutant displays no detectable staphylothrombin activity [19].

Bacteria were stored in brain heart infusion broth with 10% glycerol at −80°C. Prior to use, bacteria were allowed to grow overnight in tryptic soy broth (TSB) at 37°C in aerobic conditions, washed, and suspended in TSB.

**In Vitro Catheter Infection Model**

**Catheter Fibrin Deposition**
Sterile polyurethane quadruple lumen central venous catheters (8.5 French, 20 cm long, Arrow International) were cut into 10-mm fragments. These fragments were incubated in a suspension of either wild-type *S. aureus* Newman (WT) or *S. aureus* Newman Δcoa/vwb at an OD₆₀₀ of 1.0 on a shaking platform at 37°C for 15 minutes. After inoculation, catheter fragments were rinsed with sterile NaCl 0.9% and placed in 1 mL of freshly prepared human plasma spiked with fluorescently labeled fibrinogen (F-13191, Alexa Fluor 488 labeled, Invitrogen, Merelbeke, Belgium; final concentration, 37.5 µg/mL). Where indicated, either the LMWH enoxaparin (100 µg/mL, Sanofi-Aventis, Diegem, Belgium) or the thrombin inhibitor dabigatran (500 nM or approximately 238 ng/mL, Boehringer Ingelheim, Biberach, Germany) was added to the plasma. After 1, 2, and 24 hours, catheter fragments were washed and evaluated under an inverted fluorescence microscope (Axio-observer D1, Carl-Zeiss, Zaventem, Belgium). Images were recorded using a black and white camera (Carl-Zeiss AxioCam MRm).

**Scanning Electron Microscopy**
Catheter fragments were prepared as described above and fixed overnight in Karnovsky’s fixative (4% PFA + 2.5% glutaraldehyde in 0.1 M cacodylate buffer). Following a 2-hour period of postfixation in 2% OsO₄, samples were sequentially dehydrated in ethanol. After overnight immersion in hexamethyldisilazane, samples were coated with platina and scanned using a Jeol 7401F scanning electron microscope (Jeol Europe, Zaventem, Belgium) at 2.0 kV.

**Bacterial Retention on Catheters**
Catheter fragments were prepared as described above. After inoculation, catheter fragments were incubated in either TSB alone, TSB supplemented with prothrombin (2 µM), fibrinogen (2 mg/mL), prothrombin and fibrinogen, or plasma (50% vol/vol). Dabigatran (500 nM), argatroban (GlaxoSmithKline, Zeist, the Netherlands; 1 µM), or hirudin (Pharmion, Tiel, the Netherlands; 50 µg/mL) were added as indicated. Following overnight incubation, catheter fragments were sonicated for 10 minutes in 1 mL of sterile NaCl 0.9%, and the resulting suspension was serially diluted and plated on tryptic soy agar plates for the assessment of colony-forming units (CFUs).

To study the effect of staphylothrombin-mediated fibrin on the effect of vancomycin, catheter fragments were incubated in plasma spiked with enoxaparin (100 µg/mL), dabigatran (500 nM), or enoxaparin followed by the addition of dabigatran after 4 hours of incubation (same concentrations). Vancomycin (GlaxoSmithKline, Zeist, the Netherlands; 20 µg/mL) was added after 4 hours. Following overnight incubation, the bacterial load was assessed by quantifying CFUs after sonicing the catheter fragments and by an MTT (methylthiazolyldiphenyl-tetrazolium bromide, Sigma-Aldrich NV, Belgium) assay as...
Following sonication for 10 minutes, MTT was added to a catheter fragment with sterile NaCl 0.9% and placed in 1 mL of sterile NaCl 0.9%. Following sonication for 10 minutes, MTT was added to a final concentration of 1 mg/mL. The resulting MTT formazan was solubilized after 15 minutes in 10% dimethyl sulfoxide in acidified ethanol, and 100 µL of each sample was transferred to a 96-well plate to measure absorbance in an enzyme-linked immunosorbent assay reader at 550 nm with a 630-nm reference (A550–630).

In Vivo Catheter Infection Model
The previously described intravascular jugular vein catheter (JVC) model [23] was used with some minor modifications. Briefly, 10-week-old male BALB/c mice were anesthetized with 10% Nembutal. The cervical region was disinfected with iodinated ethanol 70%, and a right-sided cutaneous incision was made from below the masseter region to just above the clavicle. The external jugular vein was dissected and ligated at the distal end, and a sterile single-lumen polyurethane catheter was inserted through an incision. Catheters were advanced into the superior caval vein and fixed with silk stitches. Following fixation, catheters were cut and sealed, leaving approximately 5 mm of the extravascular part of the catheters. Incisions were closed in 2 layers, and the surgical region was rinsed with chlorhexidine. Animals were allowed to recuperate on a heated pad. Because there was no residual external catheter part, animals were not hindered in their movements. To assess the contribution of the presence of an intravascular catheter to the infection model, as compared to the bacteremia caused by the inoculation itself, a control group of mice underwent a sham surgical procedure in which no catheter was inserted.

Animals were fed either rodent chow supplemented with dabigatran etexilate (10 mg/g chow), or matching placebo chow (no dabigatran) beginning 12 hours after catheter insertion. In previous experiments, this chow led to dabigatran plasma concentrations of approximately 300–400 ng/mL [19, 24]. Twenty-four hours after catheter insertion, 200 µL of a suspension of S. aureus Newman WT or Δcoa/vwb (2 × 10⁶ CFU) was injected via the tail vein. When indicated, treatment with vancomycin (15 mg/kg twice daily by intraperitoneal injection) was started 24 hours after bacterial inoculation.

Five days after infection (equivalent to 4 days of antibiotic treatment), animals were euthanized, and the extravascular portion of the catheter was removed aseptically, placed in 500 µL of sterile saline, and sonicated for 10 minutes. The resulting suspensions were serially diluted and spread on tryptic soy agar plates. CFUs were counted following overnight incubation of agar plates at 37°C under aerobic conditions.

The right-sided kidneys were removed aseptically, weighed, and homogenized. Serial dilutions of the kidney homogenate were plated as described above for CFU enumeration.

All animal experiments were approved by the Ethical Committee of the University of Leuven.

Statistical Analysis
All calculations were performed using GraphPad Prism 5.0d (GraphPad Software). Values were compared using 1-way analysis of variance with the Bonferroni posttest performed when multiple values were compared. Bacterial counts were logarithmically transformed, and log-transformed values were tested for normality using the Kolmogorov-Smirnov test. Error bars in the figures and error values in the text represent mean values ± standard error of the mean. A P value of < .05 was considered statistically significant.

RESULTS

Staphylothrombin Induces Rapid Fibrin Deposition on Catheters In Vitro
Fluorescence microscopy showed a rapid deposition of fluorescently labeled fibrin on catheter fragments inoculated with S. aureus Newman WT, when incubated in enoxaparin-spiked plasma (Figure 1A). The deposition of a fibrillar fibrin structure was already evident after 1 hour of incubation and increased over time. In contrast, no increase in fluorescence was found on catheter fragments incubated in plasma spiked with dabigatran or when the Δcoa/vwb mutant was used for inoculation (Figure 1A).

Scanning electron microscopy of catheter fragments after 4 hours of incubation showed the presence of a large amount of adherent material on catheters inoculated with S. aureus Newman WT and incubated in plasma spiked with enoxaparin. This layer of adherent material consisted of cocci dispersed in a matrix of fibrillar structures and amorphous material (Figure 1B).

In the presence of dabigatran, no such adherent material was observed, and cocci were found to adhere directly to the catheter surface without any surrounding matrix (Figure 1B). Similarly, the Δcoa/vwb mutant was unable to induce this rapid increase in adherent biomaterial (Figure 1B).

Coagulases Increase Bacterial Retention on Catheters In Vitro in the Presence of Fibrinogen and Prothrombin
Viable bacteria were quantified after the overnight incubation of catheter fragments inoculated with S. aureus Newman in TSB. In the presence of fibrinogen, there was a trend toward increased retention of S. aureus (mean load [±SD], 4.23 ± 0.30 log CFU/mL vs 3.00 ± 0.23 log CFU/mL; P > .05). Addition of prothrombin had no effect on bacterial retention. In contrast, the presence of both fibrinogen and prothrombin increased bacterial retention 1000-fold (mean load [±SD], 6.04 ± 0.55 log CFU/mL vs 3.00 ± 0.23 log CFU/mL; P < .001). A similar increase in bacterial retention was observed when catheter
Figure 1. A, Staphylothrombin increases the adherence of fibrin to catheter fragments. Fluorescence microscopy imaging (original magnification, ×40) of catheter fragments inoculated with Staphylococcus aureus Newman (top and middle rows) or the Δcoa/vwb mutant (bottom row) 1, 2, and 24 hours after incubation in plasma spiked with fluorescently labeled fibrinogen in the presence of either enoxaparin (top and bottom rows) or dabigatran (middle row). Representative images from 4 different catheter fragments for each time point are shown. Dashed lines mark the inner surface of the catheter lumen. B, Scanning electron microscopy of S. aureus–inoculated catheters in the presence and absence of staphylothrombin activity. Abundant adherent biomaterial was observed on the surface of catheters inoculated with S. aureus Newman after 4 hours of incubation in enoxaparin-spiked plasma (top row, left; original magnification, ×30). This biomaterial consisted of cocci dispersed in a matrix containing fibrillar structures (upper row, right; original magnification ×5000). Both inhibition of staphylothrombin with dabigatran (middle row) and genetic absence of coagulases (Δcoa/vwb mutant; bottom row) reduced the adherent biomaterial, leaving staphylococci on the catheter surface without surrounding biomaterial.
fragments were incubated in plasma (mean load [±SD], 6.98 ± 0.42 log CFU/mL vs 3.00 ± 0.23 log CFU/mL; \( P < .001 \)). Hirudin, a thrombin inhibitor that does not inhibit staphylothrombin, did not affect bacterial retention (mean load [±SD], 6.40 ± 0.32 log CFU/mL; \( P > .05 \)). In contrast, dabigatran and argatroban significantly reduced the retention of staphylococci (mean load [±SD], 4.20 ± 0.42 log CFU/mL \( [P < .001 \text{ vs plasma} \) and 4.76 ± 0.27 log CFU/mL \( [P < .01 \text{ vs plasma} \), respectively). Semiquantitative assessment of viable bacteria on the surface of catheters by MTT staining revealed an increase in the retention of both WT and Δcoa/vwb mutant staphylococci in the presence of physiological fibrinogen concentrations (mean \( A_{550-630} \) [±SD], 0.114 ± 0.019 vs 0.021 ± 0.007; \( P < .05 \)). The presence of both prothrombin and fibrinogen led to a further increase in bacterial retention for the WT strain (mean \( A_{550-630} \) [±SD], 0.289 ± 0.028; \( P < .001 \) vs fibrinogen alone) but not for the mutant strain (mean \( A_{550-630} \) [±SD], 0.145 ± 0.028; \( P = \) not significant vs fibrinogen alone). Here again, the increased retention of WT bacteria in the presence of both fibrinogen and prothrombin was abolished by dabigatran (mean \( A_{550-630} \) [±SD], 0.120 ± 0.027; \( P = \) not significant vs fibrinogen alone, and \( P < .001 \) vs fibrinogen and prothrombin; Figure 2B).

### Genetic Inactivation or Chemical Inhibition of Staphylothrombin Increases the Susceptibility of Catheter-Grown S. aureus to Vancomycin

The addition of 500 nM dabigatran to plasma led to a significant reduction in bacterial retention after overnight incubation (mean load [±SD], 6.75 ± 0.20 log CFU/mL vs 8.36 ± 0.13 log CFU/mL; \( P < .001 \)). The effect was larger when dabigatran was present in plasma prior to catheter inoculation, although the effect of dabigatran could be observed, even when added after an initial 4-hour period of incubation in plasma (mean load [±SD], 7.28 ± 0.21 vs 8.36 ± 0.13 log CFU/mL; \( P < .01 \)). Short-term antibiotic treatment with vancomycin reduced the viable bacterial load on catheter fragments (mean load [±SD], 7.03 ± 0.13 log CFU/mL; \( P < .001 \) vs control), and the combination of vancomycin and dabigatran, either prior to bacterial inoculation (mean load [±SD], 5.11 ± 0.20 log CFU/mL; \( P < .001 \) vs dabigatran alone and vs vancomycin alone) or 4 hours after bacterial inoculation (mean load [±SD], 6.28 ± 0.11 log CFU/mL; \( P < .05 \) vs vancomycin alone, and \( P < .01 \) vs dabigatran alone) further reduced the bacterial load as compared to either treatment alone (Supplementary Figure 1).

Because preliminary experiments had shown that the CFU count of the suspension resulting from sonication of the catheters underestimated the total catheter bacterial load by about three-fourths (data not shown), we confirmed our findings by quantification of the bacterial load by MTT staining, which better reflects the total bacterial load under these conditions. The addition of dabigatran resulted in a reduction in the mean \( A_{550-630} \) (±SD) to 16.61% ± 1.2% of control levels, when added prior to inoculation, and to 71.2% ± 6.5% of control levels, when added 4 hours after inoculation (\( P < .001 \) for both comparisons). The genetic absence of staphylothrombin (Δcoa/vwb strain) reduced the bacterial load, as assessed by MTT staining, to the same extent as observed for dabigatran (mean \( A_{550-630} \) [±SD], 13.3% ± 3.8% of control levels; \( P < .001 \)). Again, the combination of vancomycin and dabigatran, either before or 4 hours after bacterial inoculation, further reduced the bacterial load as compared to either treatment alone (Figure 3).

### Contribution of Staphylothrombin to Catheter Colonization and Metastatic Infectious Complications in a Mouse Model

The bacterial load on jugular vein catheters retrieved from mice on dabigatran chow was reduced, compared with the load on catheters from mice fed a standard chow (mean load [±SD], 5.74 ± 0.18 log CFU/mL vs 7.02 ± 0.15 log CFU/mL; \( P < .05 \)), and the combination of dabigatran and vancomycin further reduced this bacterial load, compared with either treatment alone (mean load [±SD], 3.22 ± 0.33 log CFU/mL; \( P < .001 \) vs vancomycin alone, \( P < .001 \) vs dabigatran alone, and \( P < .001 \) vs control condition).

Although we did not observe a statistically significant reduction in the retention of the mutant Δcoa/vwb strain to JVCs in the absence of antibiotic treatment (mean load [±SD], 6.78 ± 0.14 log CFU/mL vs 7.02 ± 0.15 log CFU/mL; \( P = \) not significant), vancomycin treatment was more effective against infection with the mutant Δcoa/vwb as compared to the WT strain, with a larger reduction in the bacterial load (mean load [±SD], 4.88 ± 0.25 log CFU/mL; \( P < .001 \) vs no vancomycin, and \( P < .01 \) vs Newman + vancomycin; Figure 4A).

Metastatic infection is a typical feature of S. aureus catheter infections. Compared with sham-operated mice lacking an intravascular catheter, mice with a JVC had a strongly increased staphylococcal burden in the kidney tissue following induction of bacteremia with S. aureus Newman WT (mean load [±SD], 6.61 ± 0.37 vs 3.17 ± 0.51 log CFU/mL; \( P = .0002 \)).

This catheter-related increase in the bacterial load in the kidneys was reduced by both the absence and the inhibition of staphylothrombin by dabigatran (mean load [±SD], 4.71 ± 0.36 log CFU/mL \( [P < .01 \) and 4.80 ± 0.50 log CFU/mL \( [P < .01 \) vs 6.61 ± 0.37 log CFU/mL). Whereas the short-term treatment with vancomycin alone had only a small effect on the staphylococcal load in kidney tissue (5.09 ± 0.27 log CFU/mL vs 6.61 ± 0.37 log CFU/mL; \( P < .05 \)), vancomycin treatment combined with either genetic absence of coagulases (Δcoa/vwb strain; 3.15 ± 0.26 log CFU/mL) or treatment with dabigatran (2.97 ± 0.30 log CFU/mL) led to a larger reduction in bacterial load (6.61 ± 0.37 log CFU/mL; \( P < .001 \) for both comparisons). Importantly, the combination of staphylothrombin inhibition with vancomycin had an additional effect, compared with either treatment alone (\( P < .001 \) vs vancomycin alone and \( P < .01 \) vs dabigatran alone; Figure 4B).
DISCUSSION

The coagulase activity of \textit{S. aureus} has been known for over a century \cite{25}, but its role as a virulence factor in \textit{S. aureus} infections long remained controversial \cite{26, 27, 28}. Following the unraveling of the underlying molecular mechanisms and the recognition of the importance of prothrombin activation by both staphylocoagulase and von Willebrand factor–binding protein \cite{14, 16}, genetic or pharmacological strategies that target both coagulases have highlighted the role of \textit{S. aureus}–generated thrombin activity and subsequent fibrin deposition in \textit{in vitro} and \textit{in vivo} models of \textit{S. aureus} infection \cite{19, 21, 29, 31}.

We observed that catheter fragments inoculated with \textit{S. aureus} Newman WT, rather than with the isogenic Δcoa/vwb mutant, became rapidly coated with a dense fibrin matrix.

This accumulation of adherent biomaterial depended on the presence of both prothrombin and fibrinogen and was fully abrogated by the thrombin inhibitor dabigatran, which also inhibits staphylothrombin, but not by the LMWH enoxaparin. LMWHs, frequently used for thromboprophylaxis in acutely ill patients prone to catheter-related infections, target mainly factor Xa and do not impact staphylothrombin activity.

We found that this \textit{S. aureus}–triggered fibrin deposition also enhanced bacterial retention on catheter surfaces. Although the reduction of the bacterial retention was most prominent when staphylothrombin activity was blocked prior to inoculation, staphylothrombin inhibition still had an effect, albeit a smaller one, on reducing catheter bacterial load when started after fibrin structures were already present on the catheter surface.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure2.png}
\caption{A, Retention of \textit{Staphylococcus aureus} on catheter fragments. Quantitative assessment of the retention of \textit{S. aureus} Newman to catheter fragments following overnight incubation in either tryptic soy broth (TSB) with or without the addition of fibrinogen, prothrombin, or both (black squares), or in plasma (open circles). The effect of the thrombin inhibitor hirudin and the combined thrombin and staphylothrombin inhibitors dabigatran and argatroban are shown (open circles). ****\textit{P} < .001; **\textit{P} < .01. CFU, colony-forming units. B, Retention of \textit{S. aureus} on catheter fragments (MTT staining). Semi-quantitative assessment of viable bacteria on catheter fragments after overnight incubation of catheters inoculated with either \textit{S. aureus} Newman (left) or the Δcoa/vwb mutant (right) in the presence of fibrinogen (Fg), Fg and prothrombin, and Fg, prothrombin, and dabigatran. Fg increased bacterial retention in all conditions (\textit{P} < .05). The addition of prothrombin led to a further increase in bacterial retention in the wild-type strain (\textit{P} < .001) but not in the Δcoa/vwb mutant strain. This increase in bacterial retention by prothrombin was reversed by dabigatran (500 nM) (\textit{P} < .001 vs Fg and prothrombin). ****\textit{P} < .001.}
\end{figure}
This suggests that staphylothrombin-mediated fibrin does not merely mediate the initial adhesion to catheters, but also provides a protective microenvironment for bacterial growth on the catheter surface.

The capacity of staphylococci to become embedded in an extracellular matrix plays an important role in chronic and persistent infections such as endocarditis, cystic fibrosis pneumonia, and foreign body–associated infections [12, 32–34]. Although most studies of S. aureus biofilm formation have focused on the role of bacterial proteoglycans and polysaccharides, such as the polysaccharide intercellular adhesion [35–40], ex vivo studies of explanted infected catheters have revealed the presence of host proteins such as fibrinogen and fibrin as important contributors to biofilm structures [41].

Our findings demonstrate that staphylothrombin-mediated fibrin deposition serves as an additional mechanism to promote bacterial adherence to foreign bodies, thus complementing classical biofilm formation. This is in line with the findings of Katsuyama et al, who suggested that pathogen-driven fibrin formation provides a framework in which glycocalyx can be secreted in a second step [42–44].

To test the potential of staphylothrombin inhibition with or without adjunctive antibiotic treatment in vivo, we used a mouse model of JVC infection. Combined pharmacological inhibition of staphylothrombin and thrombin reduced the bacterial load on catheters in a mouse model of jugular vein catheter-related infection. *P < .05; ****P < .001.

Figure 3. Deletion or inhibition of staphylothrombin increases Staphylococcus aureus susceptibility to vancomycin in vitro. The effect of vancomycin on bacterial viability was assessed by MTT staining following incubation of catheter fragments inoculated with S. aureus Newman or Δcoa/vwb in plasma spiked with enoxaparin (control; white bars). Dabigatran was added to the plasma either prior to inoculation [dark grey bars] or 4 hours after the incubation of the catheter fragments in plasma with enoxaparin (light grey bars). *P < .05; ****P < .001.

Figure 4. A, Quantification of bacterial load on catheters in a mouse model of jugular vein catheter-related infection. *P < .05; **P < .01; ****P < .001. CFU, colony-forming units. B, Mouse catheter infection model, kidney tissue bacterial load. Quantification of the bacterial load in the kidney homogenate of mice with a jugular vein catheter 5 days after inoculation with Staphylococcus aureus Newman [black bars] or the Δcoa/vwb mutant [grey bars]. Mice were treated with dabigatran [white bars] and/or vancomycin [right bars] as indicated. *P < .05.
bacterial load on catheters. In contrast, although the mutant strain Δcoa/vwb did show a trend for lower bacterial load on the catheters, this did not reach statistical significance in our experiments. This difference between the genetic absence of staphylothrombin activity and the combined thrombin and staphylothrombin inhibition suggests that thrombin generation as a result of the activation of the physiological coagulation pathway may also contribute to the formation of fibrin deposition on inserted catheters. This is in agreement with previous observations that anticoagulation with heparin, which inhibits thrombin generation but has no effect on staphylothrombin, reduced the formation of fibrin sheaths around catheters [45–47].

Metastatic infections are a typical complication of catheter-related \textit{S. aureus} infections. In our model, absence or inhibition of staphylothrombin reduced distant infectious complications, as shown by the reduction of the bacterial load in the kidney. This may in part be the result of the higher bacterial load on the catheters, thus offering a larger reservoir for metastatic infection. However, distant infectious complications are the result of a complex interaction between bacterial adhesion and retention on one side and the effects of the humoral and cellular immune system on the other side. It was previously shown by Cheng et al that staphylothrombin enhances bacterial survival in the circulation in a sepsis model [21].

Foreign body–related \textit{S. aureus} infections typically show a reduced sensitivity to antibiotic treatment. We have previously reported that staphylothrombin-mediated fibrin shields \textit{S. aureus} from the immune system, attenuating the activation of polymorphonuclear cells and enhancing bacterial survival [19]. Here, we show that staphylothrombin-mediated fibrin not only increases bacterial retention on catheters, but also reduces the efficacy of antibiotic treatment with vancomycin. Both in vitro and in vivo, we observed a stronger reduction in viable bacteria by vancomycin in strains lacking staphylothrombin activity. Similarly, the combination of staphylothrombin inhibition by dabigatran with vancomycin led to a stronger reduction in bacterial loads on the catheters, as well as in the kidneys. This is in line with the in vitro findings of Nemoto et al, who showed that fibrinolytic activity of \textit{S. aureus} biofilms increased the efficacy of oxolinic acid in vitro and that both treatments had an additive effect [48]. Taken together, these data suggest that \textit{S. aureus}–induced fibrin deposition may contribute to the reduced susceptibility to antimicrobial therapy in foreign body–related infections and that inhibition of staphylothrombin may improve antibiotic efficacy.

In conclusion, staphylothrombin-mediated fibrin contributes to increased bacterial retention, reduced sensitivity to antibiotics, and increased metastatic potential, all of which are important clinical features of \textit{S. aureus} foreign body infections. Thus, staphylothrombin inhibition appears to be an attractive adjunctive treatment strategy in the prevention and management of \textit{S. aureus} catheter-related infections.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The postmaterials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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