Immobilization of Corn Trypsin Inhibitor on a Catheter Surface Reduces its *In Vitro* Procoagulant Properties

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Introduction

Despite advances in passivation, thrombosis remains a significant consequence of blood-contacting biomedical devices[1], such as the catheters used for percutaneous coronary interventions (PCI). Such catheters initiate coagulation via the contact factor pathway by activating factor (F) XII. Upon binding to a negatively charged surface, FXII undergoes auto-activation to FXIIa. Subsequent activation of prekallikrein by FXIIa leads to further FXIIa generation and amplification of contact-mediated coagulation. Because of its initiating role, FXIIa is an attractive target for prevention of contact pathway-initiated thrombosis induced by PCI catheters.

Corn trypsin inhibitor (CTI) is a low molecular weight (12 kDa) inhibitor of FXIIa that prolongs contact-initiated plasma clotting times[2]. Given its specificity and potency, we evaluated the utility of CTI as a surface coating to attenuate contact-initiated coagulation by PCI catheters.

Materials and Methods

The primary amine residues of CTI were modified with Traut's reagent, and then coupled to a malemide terminated polyethylene glycol (PEG) spacer. PCI catheter surfaces (Boston Scientific, Inc.) were coated with a basecoat consisting of glycidyl methacrylate and 2,2'-azobis(isobutyronitrile), as described[3] and then incubated with PEG-coupled CTI overnight at 4°C. CTI/PEG catheters were compared with untreated, PEG-treated, and ovalbumin/PEG-treated catheters. The capacity of the catheters to promote FXII auto-activation was determined using a chromogenic assay to measure FXIIa generation after incubation of catheter segments with FXII at 25C for 3-h. The ability of the catheters to inhibit FXIIa was assessed by measuring residual FXIIa activity after incubation with FXIIa. Catheter segments were incubated at 25C with 50 nM FXIIa for 1-h. Residual FXIIa was determined by chromogenic assay. The procoagulant activity of these catheters was compared by placing 2-cm segments (2-mm diameter) with 350 µL citrated human plasma. Clotting was initiated by addition of $CaCl_2$ and monitored for turbidity.

Results and Discussion

The capacity of catheter-immobilized CTI to inhibit FXIIa or attenuate FXII autoactivation was examined by chromogenic assay. Catheters with immobilized CTI reduced the activity of FXIIa by about 50% compared to untreated catheters (Figure 1). The autoactivation of FXIIa was also significantly inhibited on the CTI-treated catheters (Figure 2).

As shown in Figure 3, the procoagulant activity of CTI-coated catheters was reduced, such that the time to clot was comparable to that observed in the absence of catheter segments.

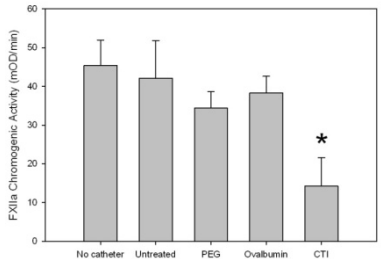

Figure 1: FXIIa activity in the presence of untreated and modified catheter segments ($n \geq 3$). * $P \leq 0.01$ compared with untreated.

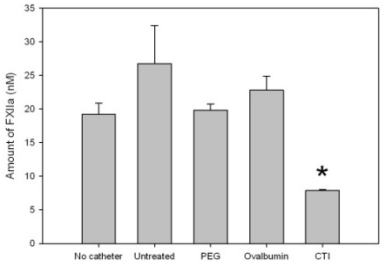

Figure 2: Auto-activation of FXII in the presence of untreated or modified catheter segments ($n \geq 3$). * $P \leq 0.05$ compared with untreated.

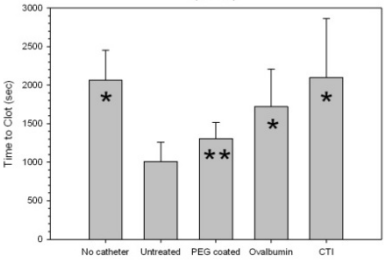

Figure 3: Plasma clotting times in the absence or presence of catheter segments ($n \geq 3$). * $P \leq 0.05$ and ** $P \leq 0.1$ compared with untreated catheter segment.

Conclusion

Immobilized CTI inhibits FXII autoactivation and FXIIa activity on the surface of PCI catheter segments. These findings suggest that CTI represents an attractive antithrombotic surface coating for PCI catheters and other blood contacting surfaces.

References

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