Comparative Factor XII Contact Activation at Hydrophilic and Hydrophobic Surfaces and Interactions with Prekallikrein and Factor XI

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Statement of Purpose: Blood coagulation resulting from contact activation due to blood – material interactions remains a challenge in the use of blood-contacting devices. The initiating step of the intrinsic pathway of plasma coagulation cascade is widely accepted to be surface contact activation of the blood zymogen FXII (Hageman Factor) into an active-enzyme form (FXIIa). This activation facilitates the assembly of a contact activation complex that is essential for enzyme amplification via reciprocal-activation prekallkrein (PK) and then FXII, as well as cascade propagation by FXIIa-mediated factor XI hydrolysis. This study seeks to clarify the differences between active FXII arising from interactions with hydrophilic vs. hydrophobic surfaces, and subsequent interactions of activated FXII with PK and coagulation factor FXI.

Methods: Glass beads were rigorously cleaned in aquaregia and piranha solutions, respectively, and rinsed with copious amount of DI water. After dry, glass beads were either used as model hydrophilic surface, or were treated with octadecyltrichlorosilane (OTS) to prepare hydrophobic surfaces[1]. FXII at 30 μg/mL was activated in a cuvette containing 100 mg of either hydrophilic or OTS-coated glass beads by mixing one hour on a hematology mixer. An *in vitro* coagulation assay was used to measure the coagulation activity of the activated FXII or interaction with other proteins as measured by coagulation time (CT), which is described elsewhere[1,2]. To assay the reactions of FXII and human PK or FXI activated by surface contact, human PK (20 μg/ml) or FXI (5μg/ml) was incubated with FXII (20μg/ml) and 100 mg of beads. Aliquots of this solution were extracted at various time points and used in coagulation assay. In addition, kallikrein generation from PK interaction with FXII contacting surfaces, and FXIa generation from FXI interaction with FXII contacting surfaces were determined using a chromogenic assay[3].

Results / Discussion:

Coagulation time of plasma with interaction of FXII and human PK. In the presence of activated FXII (FXIIa) but without excess FXII, the coagulation time of plasma is independant of [PK] regardless of concentration of FXIIa. However, coagulation time decreased significantly with increasing [PK] when excess FXII was present. These results confirm that reciprocal activation pathways with PK and FXII contributes to plasma coagulation. In the case of FXII contact acivation on hydrophilic or hydrophobic surfaces, the activated FXII added into plasma. CT of plasma for FXIIa_hydrophilic is significantly lower than that for FXIIa_hydrophobic, suggesting that more FXIIa was generated from hydrophilic surface. Subsequently, these FXII activated solutions were reacted with PK for 10 min, and then used for CT assay. Result shows that CT is shorter with FXIIa_hydrophilic than FXIIa_hydrophobic, indicating the reciprocal activation involving FXII and PK depends on surface properties (Fig. 1).

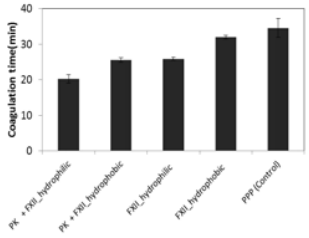

Fig. 1. CT of plasma following interaction of human PK and FXIIa activated by hydrophilic and hydrophobic surfaces.

Coagulation time of plasma following interaction of FXIIa and FXI. In the presence of activated FXII (FXIIa), CT of plasma decreased linearly with the logarithmic increase of FXIIa concentration (in case of FXI = 0 μg/ml) as seen in previous studies. When FXI was incubated with the FXIIa, FXI was converted to FXIa, and led to decreased plasma CT. However, there was no significant difference in CT as the concentration of FXIIa increased beyond 5 μg/ml. For FXIIa activated by surface contact, significantly lower CTs of plasma were found for the hydrophilic surface, and increasing FXI concentration accelerated plasma coagulation, regardless whether FXII was activated on hydrophilic or hydrophobic surfaces.

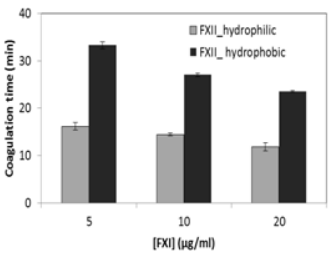

Fig. 2 CT of plasma with interaction of FXI and FXII contacting activated on hydrophilic and hydrophobic surfaces.

Chromogenic assay of kallikrein and FXIa generation. Data show that FXII activated by hydrophilic and hydrophobic surfaces converts PK to Kal at different rates (Fig. 3a), suggesting that this may lead to significant differences in the rate of coagulation. In the interaction of FXII and FXI, higher FXIa generation was found after contact with hydrophilic surfaces than hydrophobic surfaces (Fig. 3b), consistent with coagulation assay of plasma.

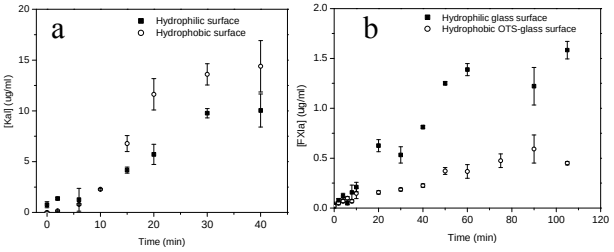

Fig. 3 Temporal changes in (a) kallikrein generation in solution of PK (20 μg/ml) and FXII contact activation, and (b) FXIa generation in solution of FXI (5 μg/ml) and FXII contact activation.

Reference:

1. Chattejee et al., Biomaterials, 2006, 27, 5643
2. Zhuo et al. , Biomaterials, 2006, 27, 4325
3. Chattejee et al., Biomaterials, 2009, 30, 4915