In vitro characterization of ACE910, a humanized bispecific antibody to factors IXa and X

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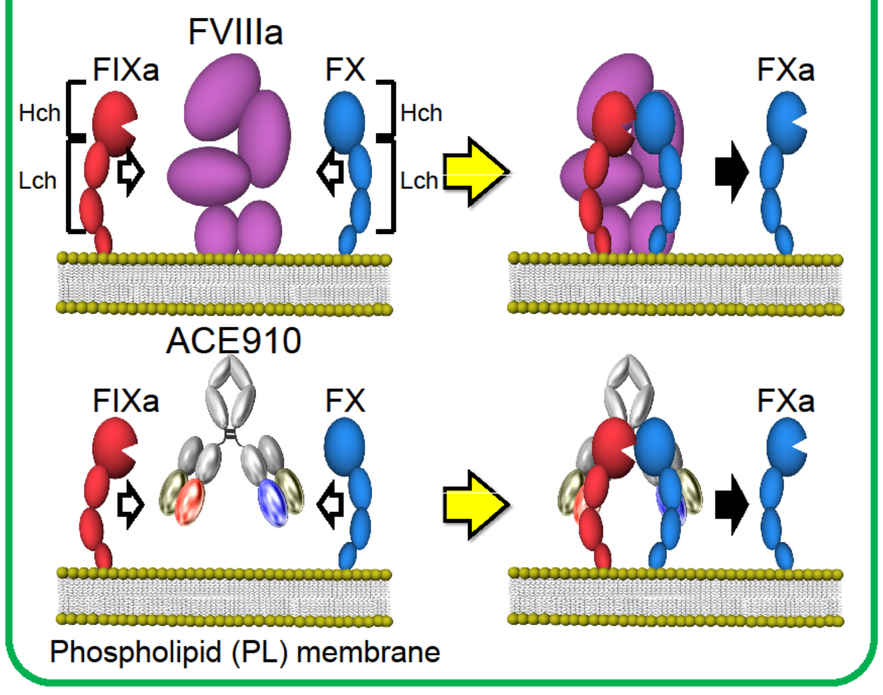
Background & Objective

- In hemophilia A, routine prophylaxis with exogenous FVIII requires frequent intravenous injections and can lead to the development of anti-FVIII alloantibodies (FVIII inhibitors).
- We developed a humanized bispecific antibody (BiAb), ACE910, that mimics the function of FVIII. After a full *in vitro* analysis, we present the functions and activity of ACE910.

Concept of FVIII-mimetic BiAb

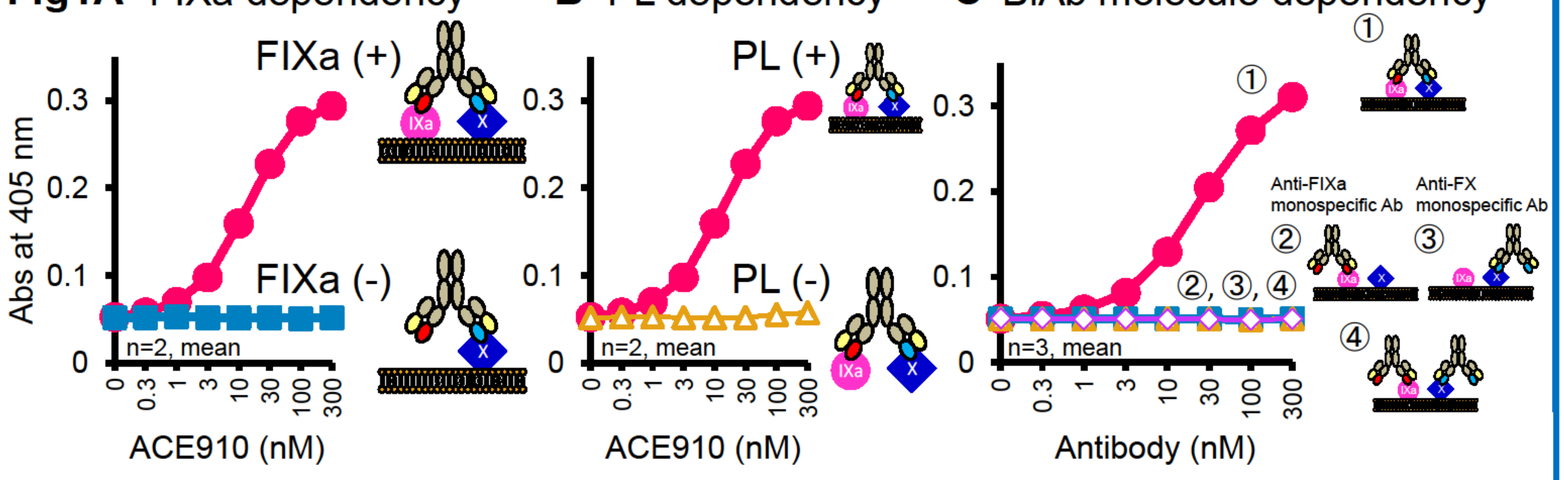


Characterization of FVIII-mimetic activity of ACE910

FXa generation assay

Method: FX activation was promoted by various antibodies in the presence or absence of PL with or without FIXa. After chromogenic substrate S-2222 had been added, the activity of generated FXa was assessed by measuring the absorbance at 405 nm.

Fig1A FIXa dependency **B** PL dependency **C** BiAb molecule dependency



Affinity analysis using Biacore

Method: Test antibodies were captured on Protein A that had been immobilized on a sensor chip. Monospecific forms of antibody were used to measure the affinity of ACE910 FIXa-arm or FX-arm to the corresponding antigen.

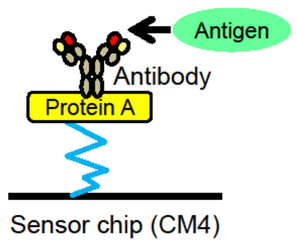


Table1 K_D values (μM)

Antigen	hFX	hFXa	hFIX	hFIXa
ACE910	1.8	0.98	1.6	1.5
FVIIIa	1～3(※1)	Not reported	Not reported	0.015(※2)

※1 J. Biol. Chem. 272, 2082–2088 (1997) ※2 J. Biol. Chem. 269, 7150–7155 (1994),

Summary1

① ACE910 functions as a cofactor that promotes the activation of FX by FIXa (Fig1A) .
② PL dependency of ACE910 suggested its activity is specific to the hemostatic site (Fig1B).
③ Bridging between FIXa and FX is required to exhibit ACE910's cofactor activity (Fig1C).
④ FXa would easily detach from ACE910 to form prothrombinase because of the low binding affinity (Table1).

= FVIII-mimetic activity

Potency of FVIII-mimetic activity of ACE910 in coagulable reaction

Kinetics analysis

Method: The rate of FXa generation in the presence of ACE910 or FVIIIa was determined by FXa generation assay. The data were fitted to Michaelis-Menten equation to calculate kinetics parameters.

K_m: Michaelis-menten constant
V_{max}: Maximum velocity
k_{cat}: Catalytic rate constant
k_{cat}/K_m: Catalytic efficacy

Table2 Kinetics parameters

Condition	K_m (μM)	V_{max} (nM/min)	k_{cat} (/min)	k_{cat}/K_m	(x-fold)
FIXa+FX+PL	0.0986	0.0257	0.000643	0.00652	(1)
+ ACE910	0.00505	2.88	2.88	570	87400
+ FVIIIa	0.0195	126	126	6460	991000

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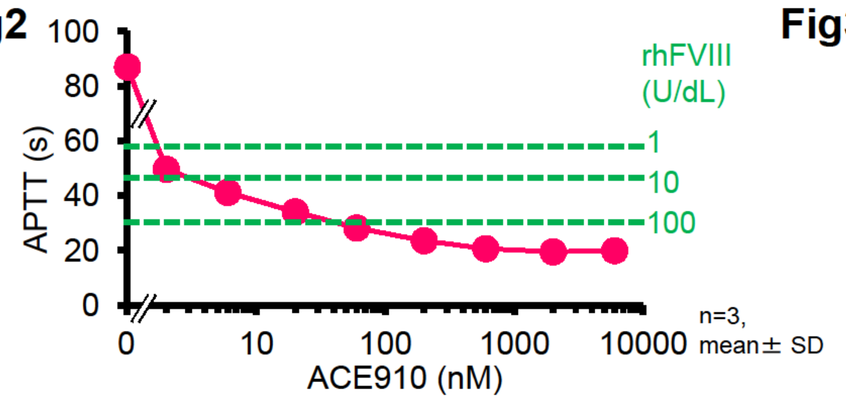
n=3, mean

APTT assay

Method: ACE910 or rhFVIII (Kogenate FS, Bayer) was added to FVIII-deficient human plasma (George King). APTT assay was performed using Thrombocheck APTT-SLA (Sysmex).

Fig2

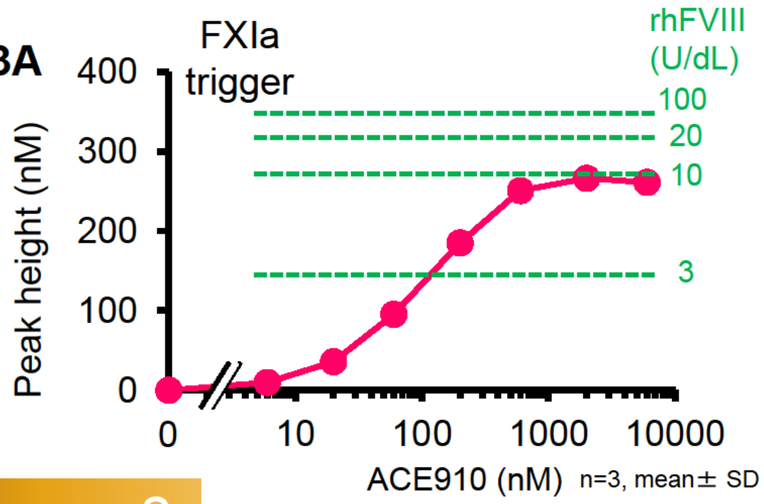


Much shorter APTT with ACE910 beyond the level achieved with 100 U/dL FVIII (=normal level) can be explained by the additional time FVIII requires to be activated by thrombin.

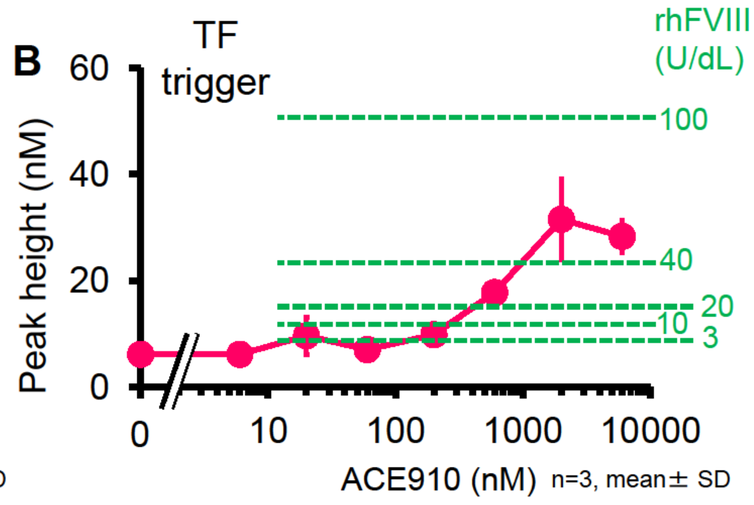
TG assay

Method: Thrombin generation (TG) used two kinds of triggering solutions: 0.16 nM human FXIa and 20 μM PL as the FXIa trigger, and PPP-Reagent LOW (Thrombinoscope) as the TF trigger

Fig3A



B



Summary2

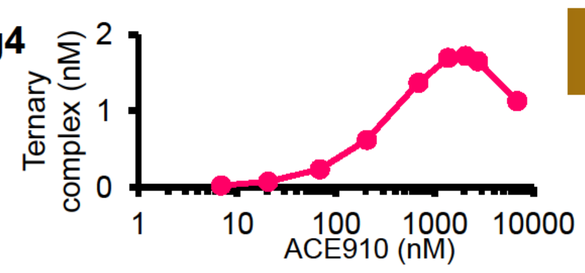
ACE910 exerted an equivalent activity to 10 U/dL or above of FVIII (Fig 3).

Simulation of FIX-ACE910-FX complex concentration in plasma

Parameters: Plasma FIX→ 89 nM K_D for FIX→ 1.6 μM
FX→136 nM FX→ 1.8 μM

Equation: $$[complex] = \frac{([Ag_t]+[Ma_t]+K_D)-\sqrt{([Ag_t]+[Ma_t]+K_D)^2-4\cdot[Ag_t]\cdot[Ma_t]}}{2}$$

Fig4



ACE910's cofactor activity corresponded with the calculated level of FIX-ACE910-FX ternary complex, underwriting the bridging hypothesis (Fig 1C).

Conclusion

ACE910 functions as a FVIII-mimetic cofactor that can work in the coagulation cascade.

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