PB0536: Oral Factor XIIa inhibitor KV998086 protects against $FeCl_3$ induced thrombosis in mice.

Authors: Allen Clermont[1], Freya Pinckney[2], Marza Hill[1], Sally L. Hampton[2], Edward P. Feener[1]

KalVista Pharmaceuticals: [1] Cambridge, MA USA, [2] Porton Down, UK

INTRODUCTION

Factor XIIa (FXIIa) has been proposed as a potential therapeutic target for novel anticoagulants that may prevent thrombosis without increased bleeding risk. FXIIa is a trigger for the intrinsic coagulation pathway, contributing to thrombosis. However, FXIIa is not required for normal hemostasis[1]. Previous studies have shown that FXII deficiency and antibody mediated FXIIa inhibition in mice provide significant protection against thrombosis without increased bleeding[2,3,4]. In thrombosis studies on non-human primates, antibody mediated inhibition of FXIIa reduced thrombus formation and platelet deposition[5,6]. We have used structure-based drug design to discover potent, selective and orally small molecule FXIIa inhibitors, including KV998086.

AIM

This study evaluates the effects of the novel oral FXIIa inhibitor KV998086 on $FeCl_3$-induced arterial thrombosis in mice.

METHOD

Enzyme activity: KV998086 effects on FXIIa enzyme activity were assessed using a fluorogenic assay with H-D-Pro-Phe-Arg-AFC as substrate. Effects of KV998086 on enzyme activity for additional coagulation pathway factors, FXIa, FXa and thrombin were measured to determine inhibitor selectivity.

Pharmacokinetics: Male Sprague Dawley rats (n=2) were given KV998086 (5 mg/kg) by oral gavage. Blood samples were taken from a lateral tail vein and stored in citrate coated vials. For mouse PK, male C57bl6 mice (n=18) were given KV998086 by oral gavage (45 mg/kg). Blood samples were taken from the inferior vena cava. Quantification of plasma concentration was performed by LC-MS/MS using a KV998086 reference sample.

Activated partial thromboplastin time (aPTT) and prothrombin time (PT): aPTT and PT were assessed using the Ceveron Alpha automated coagulation analyzer (Technoclone). C.K. Prest Reagent (Stago, 00597) was used as the aPTT intrinsic pathway activator and STA-NeoPTimal (Stago, 12006) was used as the PT extrinsic pathway activator. KV998086 was added to the plasma prior to activation and assays were run as per manufacturer's instructions.

Thrombin Generation Assay (TGA): Thrombin generation was assessed using a Z-G-G-R-AMC substrate containing 15mM $CaCl_2$ (Diapharma, 5006235). Coagulation was stimulated using 50µg/mL long chain polyphosphate, PolyP (Kerafast, EUI004) in TGA buffer (Diapharma, 5006360). Thrombin generation was monitored for 2 hours at 37ºC.

Thrombosis models: Carotid artery thrombosis in mice was initiated by 3.5% $FeCl_3$ patch for 3 minutes. A Doppler flow probe (Transonic TS420) was used to monitor blood flow for up to 30 minutes. Time to occlusion (TTO) was determined in WT and FXII KO mice and WT with KV998086 administered via pumps or oral gavage. WT mice were administered vehicle or 5.8 mg/kg/day KV998086 by micro-osmotic pump (Alzet, 1003D) implanted subcutaneously 48 hours prior to $FeCl_3$ injury. For oral studies, mice were administered two gavages of 45mg/kg KV998086 over a 24h period prior to $FeCl_3$ injury.

RESULTS

Figure 1: Coagulation cascade scheme

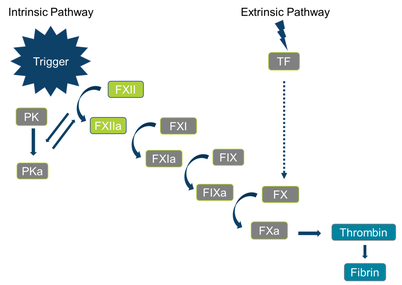


Table 1. Potency and selectivity

	KV998086
Enzyme	**IC_{50}**
Factor XIIa	7.2 nM
Factor XIa	>40 µM
Factor Xa	>40 µM
Factor VIIa	>10 µM
Plasma Kallikrein	>40 µM
Plasmin	>40 µM
Thrombin	16 µM
Trypsin	>40 µM
Tissue Kallikrein 1	7.9 µM

Table 1: KV998086 protease selectivity
IC_{50} (half maximal inhibitory concentration) values for KV998086 for Factor XIIa and a panel of serine proteases in isolated enzyme kinetic substrate cleavage assays.

Figure 2 Pharmacokinetic profile of KV998086 in mouse

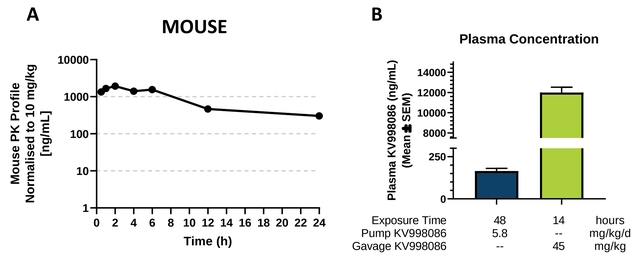


Figure 2. Pharmacokinetic profile and Plasma exposure
A) Pharmacokinetic profile for a single gavage of KV998086 in mouse. PK profile is normalized to a 10 mg/kg dose. Cmax occurs at 2 hours with a plasma concentration at 24 hours equal to 16% of Cmax. B) Plasma exposure for studies with osmotic pump (5.8 mg/kg/d) at 48 hours and for 2 oral gavage administrations by 14 hours.

Figure 3: Effect of KV998086 on $FeCl_3$-induced arterial thrombosis

A. Percentage of baseline flow post $FeCl_3$ injury

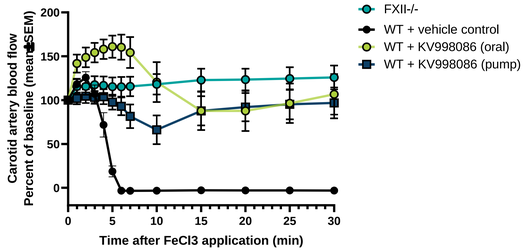


Figure 3A: Administration of KV998086 by oral gavage at 45 mg/kg or SC osmotic pump at 5.8 mg/kg/d protects-against $FeCl_3$-induced thrombosis compared to Factor XII knockout mice.

B. Time to occlusion

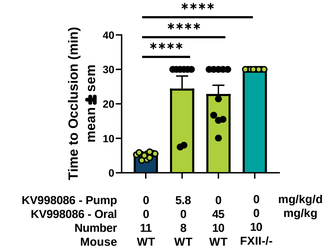


Figure 3B: $FeCl_3$-induced time to occlusion in the carotid artery is prolonged by administration of KV998086 compared to WT vehicle control mice

C. In Vivo aPTT

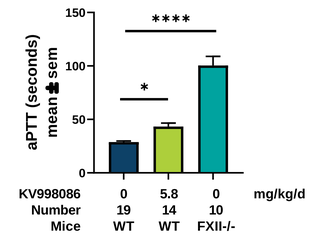


Figure 3C: Activated partial thromboplastin clotting time (aPTT) in mouse is prolonged by KV998086 administered via osmotic pump at 5.8 mg/kg/d in WT mice.

Figures 4 and 5: Effect of KV998086 on thrombin formation and aPTT in human plasma

Dose response of KV998086 in thrombin generation assay (TGA)

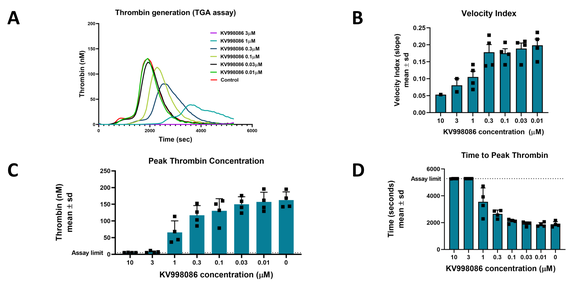
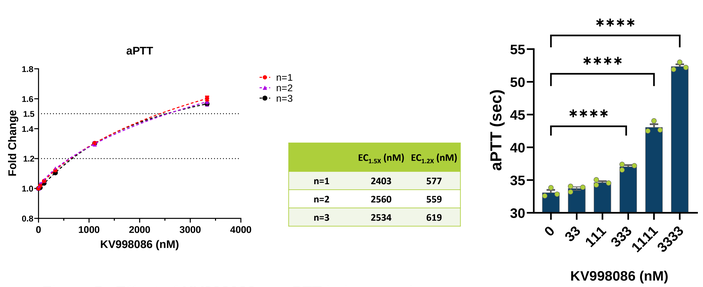


Figure 4. Dose response of KV998086 in the TGA
A) Effect of KV998086 upon thrombin generation in human plasma pre-treated with concentrations of 0.01 to 10 µM compared to vehicle alone. B-D) Analysis from A)

Effect of KV998086 on aPTT



	$EC_{1.5x}$ (nM)	$EC_{1.2x}$ (nM)
n=1	2403	577
n=2	2560	559
n=3	2534	619

Figure 5. Effect of KV998086 on aPTT in human plasma.
Dose response of KV998086 upon activated partial thromboplastin time in human plasma. KV998086 exhibits an $EC_{1.2x}$ of 585nM and an $EC_{1.5x}$ of 3.18µM

SUMMARY & CONCLUSIONS

- To our knowledge, this is the first report of a potent and selective FXIIa inhibitor with high oral availability shown to inhibit thrombosis in mice.
- KV998086 is a selective small molecule inhibitor of FXIIa with high bioavailability and prolonged plasma exposure in rodent models.
- FXII knockout mice were fully protected against $FeCl_3$ induced arterial thrombosis compared to WT mice.
- Both oral and subcutaneous administration of KV998086 were protective against $FeCl_3$ induced thrombosis.
- In human plasma, KV998086 blocked PolyP induced thrombin formation in a dose responsive manner.
- KV998086 prolonged aPTT with an $EC_{1.2x}$ = 585nM without affecting PT ($EC_{1.5x}$ > 33µM). (data not shown)
- In conclusion, we have demonstrated that small molecule FXIIa inhibitors may provide a novel class of antithrombotic therapeutics.

DISCLOSURE

ACC, FP, MH, SLH, EPF are employees of KalVista Pharmaceuticals.

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CONTACT INFORMATION

Allen Clermont,
Director, In Vivo Pharmacology,
KalVista Pharmaceuticals.
Allen.Clermont@kalvista.com