PB0536: Oral Factor Xll inhibitor KV998086 protects against FeCl₃ induced thrombosis in mice.
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INTRODUCTION

Factor Xlla (FXlla) has been proposed as a potential therapeutic target for novel anticoagulants that may prevent thrombosis without increased bleeding risk. FXlla is a trigger for the intrinsic coagulation pathway, contributing to thrombosis. However, FXlla is not required for normal hemostasis¹. Previous studies have shown that FXI deficiency and antibody mediated inhibition of FXlla in mice provide significant protection against thrombosis without increased bleeding²,³. In thrombosis studies on non-human primates, antibody mediated inhibition of FXlla reduced thrombus formation and platelet deposition⁴,⁵. We have used structure-based drug design to discover potent, selective and orally small molecule FXlla inhibitors, including KV998086.

AIM

This study evaluates the effects of the novel oral FXlla inhibitor KV998086 on FeCl₃-induced arterial thrombosis in mice.

METHOD

Enzyme activity: KV998086 effects on FXlla enzyme activity were assessed using a fluorogenic assay with H-D-Pro-Arg-APC as substrate. Effects of KV998086 on enzyme activity for additional coagulation pathway factors, FXlla, 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 cholate coated vials. For mouse PK, male C57Bl/6 mice (n=18) were given KV998086 by oral gavage (45 mg/kg). Blood samples were taken from the inferior vena cave. Quantification of plasma concentration was performed by LC-MS/MS, using a KV998086 reference sample.

Activated partial thromboplastin time (aPTT) and prothrombin time (PT) were measured using the Chroma LH automated coagulation analyzer (Technicon, Tarrytown, NY). C. Kryst Prez Regan (Stago, 00597) was used as the aPTT intrinsic pathway activator and STA NA-ProTm (Stago, 12006) was used as the PT extrinsic pathway activator. KV998086 was added to the plasma prior to activation and analyses were run as per manufacturer’s instructions.

Thrombin Generation Assay (TGA): Thrombin generation was assessed using a JenaCo® R-Pro®, substrate containing 1.0 M CaCl₂ (Dyraphe, 500023). Coagulation was stimulated using 50μg/ml long chain phospholipid, PolyP (Kalexal, EU004) in TGA buffer (Dyraphe, 500360). Thrombin generation was monitored for 2 hours at 37ºC.

Thrombosis models: Carotid artery thrombosis in mice was initiated by 3.5 mg/kg FXIIa, patch for 3 minutes. A Doppler flow probe (Transonic T5400) was used to monitor blood flow for up to 30 minutes. Time to occlusion (TOO) was determined in WT and FXII-/- 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 FeCl₃ injury. For oral studies, mice were administered two gavages of 45mg/kg KV998086 over a 24 hour period prior to FeCl₃ injury.

RESULTS

Figure 1: Coagulation cascade scheme

Figure 2: Pharmacokinetic profile of KV998086 in mouse

Table 1: Potency and selectivity

Table 1: KV998086 probe selectivity

Figure 3: Effect of KV998086 on FeCl₃-induced arterial thrombosis

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

DISCUSSION

To our knowledge, this is the first report of a potent and selective FXlla inhibitor with high oral availability shown to inhibit thrombosis in mice.

KV998086 is a selective small molecule inhibitor of FXlla with high bioavailability and prolonged plasma exposure in rodent models.

KV998086 blockade of FXlla induced thrombin formation in a dose responsive manner.

KV998086 prolonged aPTT with an EC₅₀ > 585nM without affecting PT (EC₅₀ > 33μM).

In conclusion, we have demonstrated that small molecule FXlla inhibitors may provide a novel class of antithrombotic therapeutics.

REFERENCES


CONTACT INFORMATION

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