Oral factor XIIa inhibition blocks angiotensin converting enzyme inhibitor induced angioedema in mouse

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Background

- ACEi-AE is a complication associated with ACEi treatment. [1]
- The reported incidence rate is 0.1–0.7% with ~ 50% of cases occurring in the first week of treatment. [1, 2]
- Studies have implicated uncontrolled plasma kallikrein activity, leading to the release of bradykinin, and resultant permeability and inflammation. [3-5]
- Targeting FXII of the contact system has been shown to reduce vascular leakage in pre-clinical mouse models of AE. [6]
- Administration of garadacimab reduced the number of monthly attacks in patients with HAE by inhibiting FXII generation of plasma kallikrein. [7]
- Oral inhibitors of activated FXII may provide treatment for ACEi-AE and other forms of angioedema.

Purpose

This study evaluated the effects of the oral FXIIa inhibitor KV998086 on ACEi-induced vascular leakage in mice.

Methods

ACEi (Captopril)-induced vascular permeability in mice:
A cannula was inserted in the right jugular vein and connected to a 100µL Hamilton syringe. Captopril was infused at 2.5 mg/kg followed by Evans blue dye at 30 mg/kg. After 30 minutes of circulation time, the mouse was sacrificed and larynx, trachea and colon were collected.

Evans-blue assay:
Tissues are weighed and incubated in formamide at 72ºC. Samples were centrifuged and supernatant loaded on a 48 well plate. Samples were scanned at 620 -740 nm for absorbance on a plate reader. Evans blue concentration was calculated by a standard curve and normalized by dry weight of the tissue (ng/mg tissue).

Mice:
Age matched wildtype (WT) C57bl/6 mice were obtained from Jackson Labs. Factor XII KO were backcrossed with C57bl/6 for 9 generations resulting in genetic purity > 99%.

Dextran Sulfate (DXS) plasma HK cleavage assay:
Whole human plasma was pre-treated with KV998086 for 15 minutes. DXS (6.25 µg/ml) was added to each sample to stimulate contact activation. PKa protein levels were quantified using a PKa primary antibody, anti-PKa (AB44932) with a 1:100 dilution using chemiluminescence (ProteinSimple).

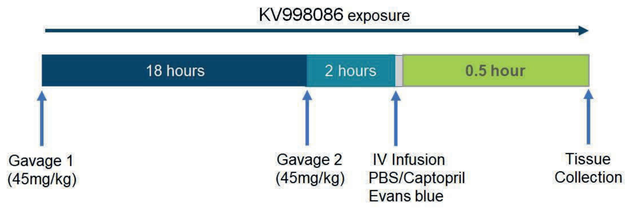
KV998086

- KV998086 is a potent and orally available FXIIa inhibitor (human IC_{50}=10nM, mouse IC_{50}=8.5nM)
- >4000 fold more selective for FXIIa than closely related serine proteases.

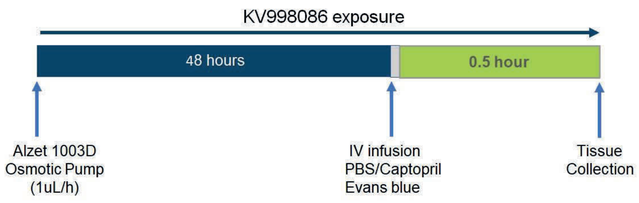
	KV998086
Enzyme	IC50 nM
FXIIa	10
Selectivity	
FXa	>40000
Plasma Kallikrein	>40000

In Vivo Studies

Oral KV998086 administration:
KV998086 was prepared in 10% DMSO, 10% Cremaphor El, 80% Water at 4 mg/mL. Each mouse received 2 oral doses of vehicle or KV998086 (45mg/kg) at 18 and 2 hours prior to captopril infusion.

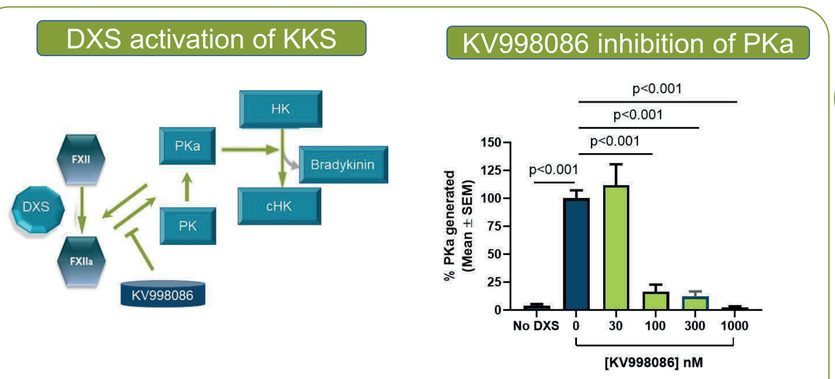


Osmotic pump KV998086 administration:
KV998086 was prepared in 20% cyclodextrin at 6 mg/mL. Alzet 1003D pumps were implanted SC in the flank of each mouse at 48 hours prior to captopril infusion.



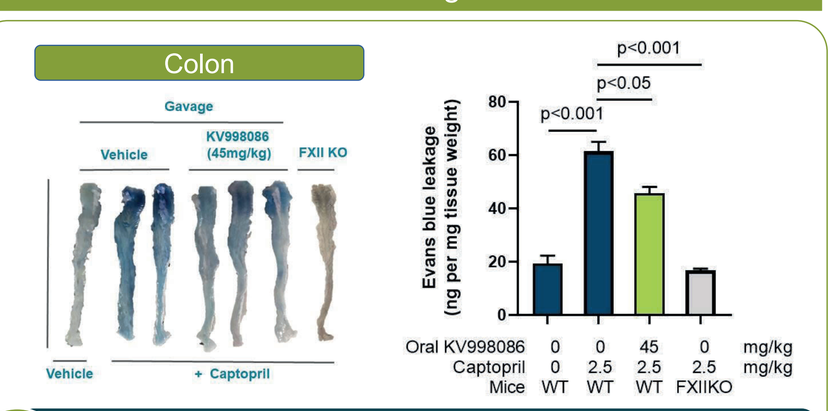
Results

Figure 1: KV998086 inhibition of dextran sulfate (DXS) stimulated KKS activation in whole human plasma



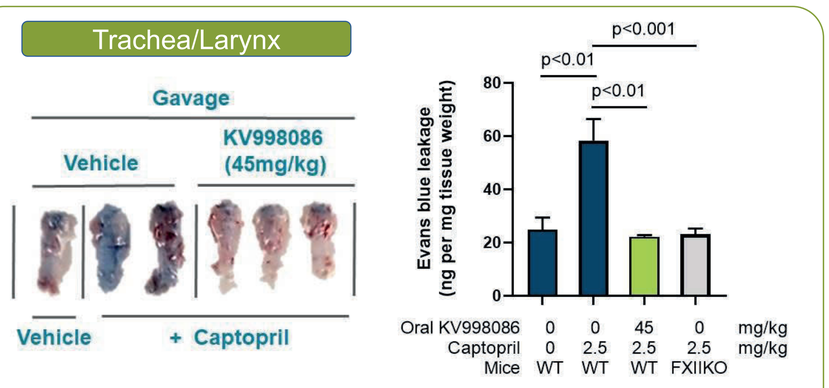
FXIIa inhibition blocked the generation of plasma kallikrein in whole human plasma with an IC_{50} = 59.8 nM

Figure 2: Effect of oral KV998086 upon captopril-induced Evans blue leakage in colon



Orally administered KV998086 reduced ACE inhibitor stimulated leakage in colon

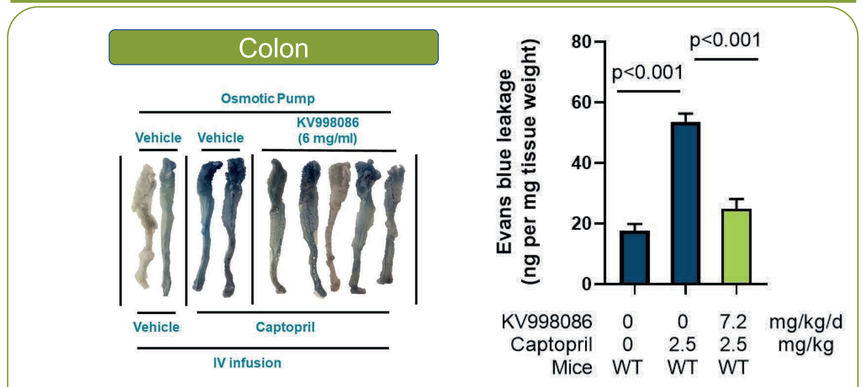
Figure 3: Effect of oral KV998086 upon captopril-induced Evans blue leakage in trachea/larynx



Orally administered KV998086 reduced ACE inhibitor stimulated leakage in trachea/larynx

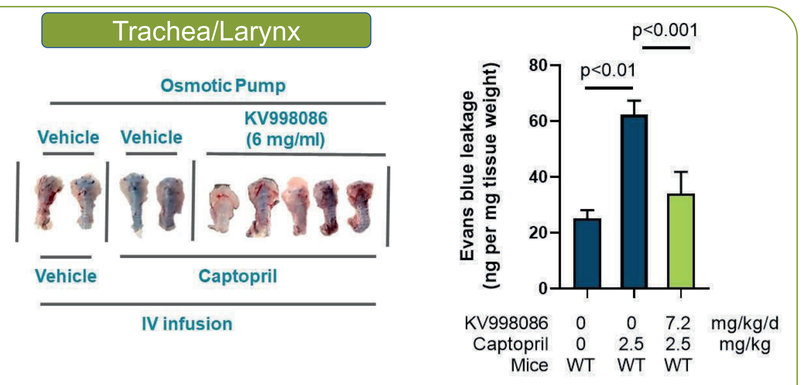
Results

Figure 4: Effect of 48 hour osmotic pump KV998086 upon captopril-induced Evans blue leakage in colon



Continuous SC delivery of FXIIa inhibitor KV998086 reduced ACE inhibitor stimulated leakage in colon

Figure 5: Effect of 48 hour osmotic pump KV998086 upon captopril-induced Evans blue leakage in trachea/larynx



Continuous SC delivery of FXIIa inhibitor KV998086 reduced ACE inhibitor stimulated leakage in trachea/larynx

Conclusions

- KV998086 blocked the generation of plasma kallikrein (PKa) in whole human plasma with an IC_{50}=59.8nM
- Factor XII KO mice are protected against captopril induced bradykinin-mediated vascular leakage
- Orally administered FXIIa inhibitor KV998086 reduced ACE inhibitor stimulated leakage in colon and larynx/trachea
- Osmotic pump administration of KV998086 for 48 hours suppressed captopril induced leakage in colon and larynx/trachea
- These data indicate that the inhibition of activated FXII by oral administration may provide prophylactic prevention of ACE inhibitor associated angioedema and potentially other forms of AE such as HAE

References

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Conflict of interest disclosure

All authors are employees of KalVista Pharmaceuticals