ePDS: 350

Selective Factor XIIa inhibitor KV998083 protects mice against captopril induced vascular leakage and cleavage of high molecular weight kininogen.

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Background

- Hereditary angioedema (HAE) is a genetic disorder that causes recurrent episodes of tissue swelling in the skin and mucosal membranes
- Studies have implicated uncontrolled plasma kallikrein activity as a primary cause of attacks
- Contact system activation generates bradykinin which increases vascular permeability and inflammation
- FXIIa inhibition may provide a novel therapeutic target to prevent the activation and amplification of the contact system in HAE

Objective

This study evaluates the effects of FXII gene knockout and the FXIIa inhibitor KV998083 on angiotensin converting enzyme (ACE) inhibitor captopril-induced vascular and bradykinin-mediated vascular hyperpermeability in mice

Methods

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

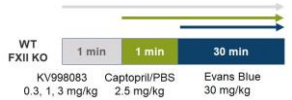
Evans-blue assay:
Tissues are weighed and incubated in formamide at 72ºC. Samples are centrifuged and supernatant loaded on a 48 well plate. Samples are scanned at 620 -740 nm for absorbance on a plate reader. Evans blue concentration is calculated by a standard curve and normalized by dry weight of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

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%.

Icatibant administration:
Icatibant (Sigma: HOE-140) or vehicle alone was loaded into Alzet osmotic pumps (1003D) and subcutaneously implanted in WT mice. After 24 hours, mice received an IV infusion of captopril and Evan's blue.

Methods

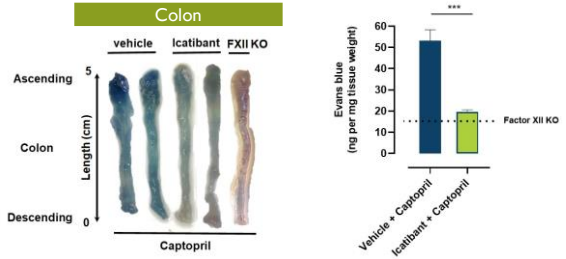
KV999083 administration:
KV998083 was infused at 0.3, 1.0 and 3.0 mg/kg by IV at 1 minute prior to captopril infusion.



Dextran Sulfate (DXS) plasma HK cleavage assay:
Citrated blood was collected from WT mice at 30 minutes after IV infusion of KV998083 at 1 mg/kg or vehicle alone. DXS (6.25 µg/ml) is added to each sample to stimulate contact activation. Samples are loaded onto a WES System (ProteinSimple) plate. HK protein levels are quantified using an HK primary antibody, anti-HK (Santa Cruz SC-25887) with a 1:100 dilution.

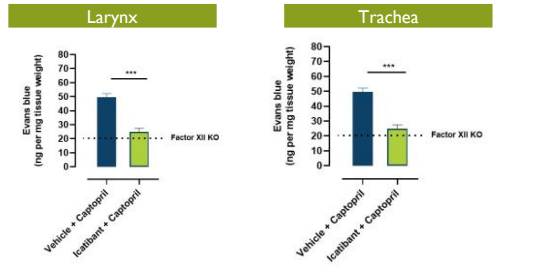
Captopril-induced plasma HK cleavage assay:
Citrated blood was collected from WT mice at 30 minutes after IV infusion of captopril at 2.5 mg/kg or vehicle. HK protein levels were measured as above.

Effect of Icatibant upon captopril-induced Evans blue leakage in colon



Captopril induced leakage is mediated by bradykinin 2 receptor

Effect of Icatibant upon captopril-induced leakage in larynx and trachea



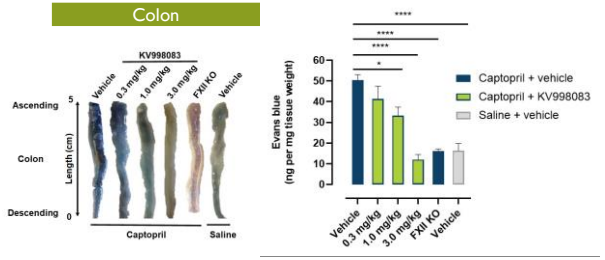
Icatibant decreases captopril induced leakage to levels similar to Factor XII KO mouse

KV998083 Selectivity

Enzyme	Species	IC_{50} (nM)
FXIIa	Mouse	43.1
PKa	Mouse	1595
FXIIa	Human	36.9
KLK1	Human	1700
FXa	Human	>40,000
FXIa	Human	>40,000
Thrombin	Human	>40,000
Plasmin	Human	29,453
tPA	Human	>40,000
Matriptase	Human	>40,000

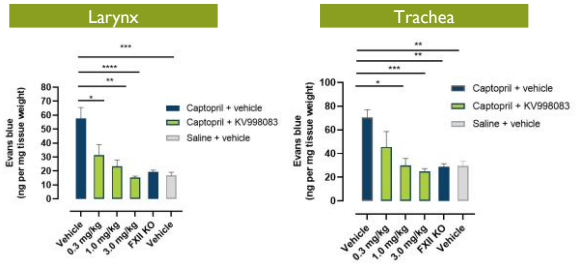
KV998083 is potent and selective inhibitor of Factor XIIa

Effect of KV998083 on captopril-induced Evans blue leakage in colon



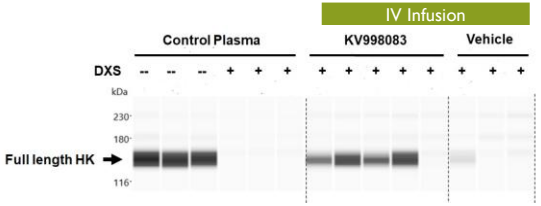
Administration of KV998083 inhibits captopril induced leakage in a dose responsive manner

Effect of KV998083 on captopril-induced Evans blue leakage in larynx and trachea



KV998083 inhibits captopril induced leakage equivalent to levels in Factor XII KO mice

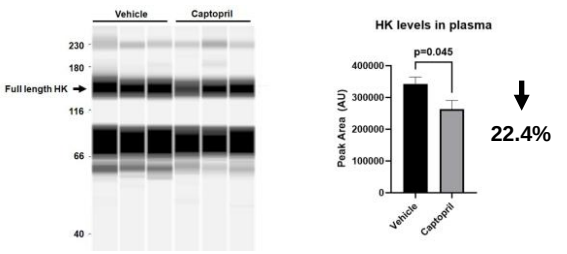
Factor XIIa inhibitor KV998083 protects against HK cleavage in ex vivo DXS stimulated plasma



HK remaining: 47.4% 6.2%
p=0.034

KV998083 at 1 mg/kg I.V. protects HK from cleavage in mouse plasma exposed to DXS

HK levels in mouse plasma are reduced following captopril infusion at 30 minutes



Decrease in plasma HK levels indicates that captopril increases HK consumption

Conclusions

- Factor XII KO mice were protected against captopril induced bradykinin-mediated vascular leakage
- KV998083 administration suppressed captopril induced leakage in a dose responsive manner (0.3, 1, and 3 mg/kg)
- Captopril-induced leakage with KV998083 administration at 3 mg/kg was not different from FXII KO levels
- KV998083 achieves protection of HK in plasma
- FXIIa inhibition may provide prophylactic prevention of bradykinin-induced angioedema

Disclosures: ACC, NM, HJE, SAW, SJP, EJD, SLH, EPF: KalVista Pharmaceuticals