KVD900 protects high molecular weight kininogen (HK) from ex vivo plasma kallikrein-mediated cleavage in plasma from patients with hereditary angioedema (HAE): Results from a semi-automated capillary-based immunoassay.

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Introduction

Decreased levels of full-length HK and increases in the heavy and light chains of HK have been proposed as plasma biomarkers for HAE attacks and for the evaluation of therapeutic interventions that inhibit plasma kallikrein (PK). Measurements of plasma HK have largely relied on traditional western blot immuno-assays, which are labor intensive and associated with high inter-assay signal to background variability. We have developed a semi-automated capillary-based immunoassay for the quantification of HK in undiluted human plasma and have compared this method to traditional western blot analysis.

Objective

To develop a semi-automated method to quantify HK (full-length) in plasma and use this method to evaluate the effects of a novel, orally available PK inhibitor, KVD900 on the protection of PK-mediated HK cleavage in undiluted HAE and control plasma in an ex vivo assay.

Methods

Ex-vivo whole plasma HK cleavage assay:

HK cleavage in undiluted citrated human plasma was induced by contact system activation with dextran sulfate (DXS, Sigma #31395-10G; 6.25 µg/ml) on wet ice. Pooled normal (CONTROL) human plasma (VisuCon-F Frozen Normal Control plasma) was purchased from Affinity Biologicals Inc. A working stock of 10mM KVD900 in DMSO was prepared and diluted in 1X PBS to the respective final concentrations described. HAE plasma was obtained from HAE subjects (n=6) and C1-inhibitor deficiency was confirmed by western blotting. HK was quantified in plasma by either western blotting or capillary-based immunoassay.

Western blotting:

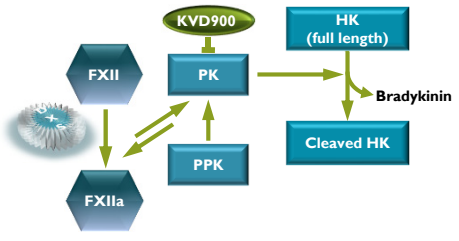
SDS-PAGE gel electrophoresis was done using 7.5% Criterion TGX Precast gels (Bio-rad). Transfer was made onto Immobilon-FL PVDF membrane. Image analysis was performed using the LICOR imaging system. Mouse monoclonal anti-human HK antibody (MAB15692, R&D systems) was used for traditional immunoblotting.

Capillary-based immunoassay on the WES System (ProteinSimple):

Preparation of samples: Combine one part 5× fluorescent master mix with four parts of the 1:200 plasma sample. Vortex to mix. Heat the samples + fluorescent master mix and the biotinylated ladder at 95°C for 5 minutes, vortex, and load onto the WES plate. Monoclonal anti-human HK antibody was used for this chemiluminescence-based detection method using the Wes System (ProteinSimple).

Analysis: Collect the peak area measurement obtained in the Compass software (cbz file) for the full-length HK molecular weight of the respective time-point sample with DXS-induced activation. The peak area is defined as the area calculated for the spectral peak profile for HK (as shown in the figure 4B). To measure the plasma kallikrein inhibition by KVD900, the percent full-length HK detected was calculated.

Figure 1: Dextran sulfate mediated activation of the contact system



Results

Figure 2: Standard curve of single chain human HK purified from human plasma run on the capillary-immunoblotting platform

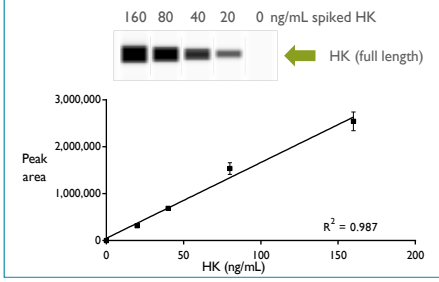


Table 1: Potency & Selectivity of KVD900

KVD900	Ki (nM)
Plasma Kallikrein	3.02
Selectivity vs PK	**Fold**
Tissue Kallikrein	>6000
Factor XIa	>6000
Factor XIIa	>6000
Plasmin	>6000
Thrombin	>6000
Trypsin	>6000

Figure 3: IC_{50} of KVD900 and purified C1-Inh for PK activity in human plasma (diluted 1:4)

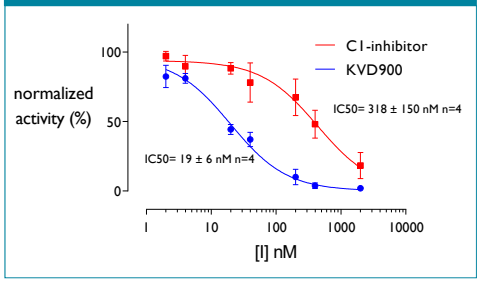


Figure 4: Dose response of KVD900 on full length HK levels in undiluted healthy control plasma stimulated ex vivo with dextran sulfate.

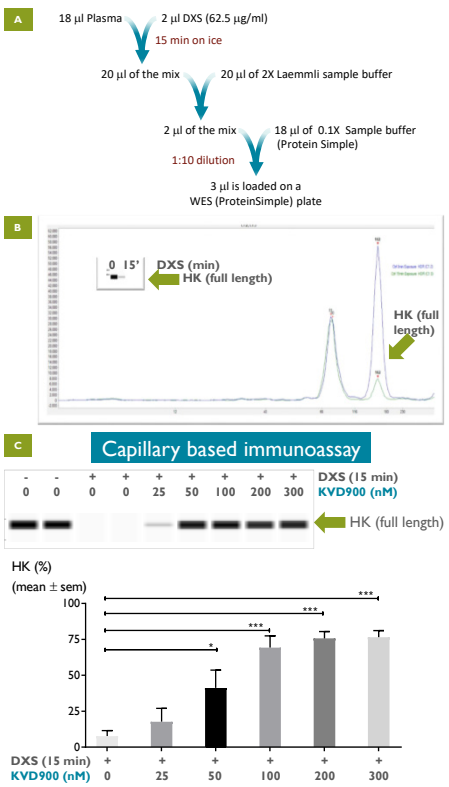


Figure 4: cont'd

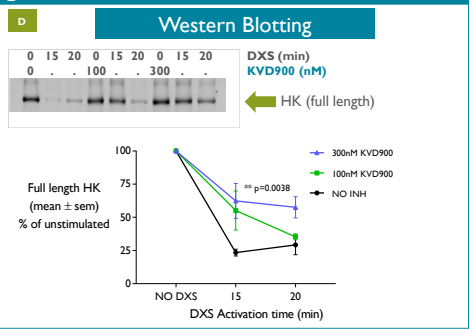


Figure 5: Time course of dextran sulfate-activated cleavage of HK in HAE whole plasma

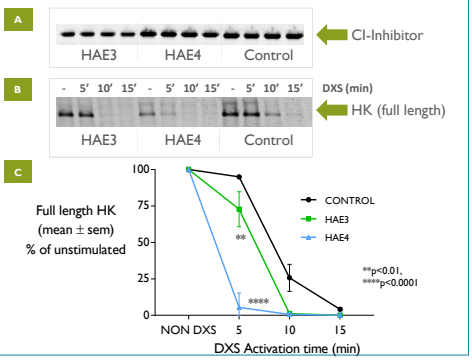
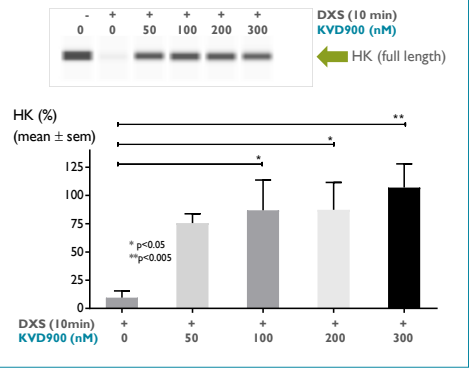


Figure 6: Dose response of KVD900 on full length HK levels in dextran sulfate-activated HAE plasma.



Summary & Conclusion

KVD900 is a potent and selective orally-available plasma kallikrein inhibitor.

KVD900 provides dose dependent protection against HK cleavage in both HAE and healthy control plasma stimulated with dextran sulfate.

A capillary-based immunoassay provides a rapid (<3hrs) and semi-automated method to quantify HK in human plasma: 5-fold faster than traditional western blotting with a linear detection range of HK at physiological plasma concentrations.

KVD900 is currently being studied in a Phase 1 trial in healthy volunteers. Plasma-based assays will be used to assess its pharmacodynamic effects.

Conflict of Interest Disclosures

EPF, NM, PAR, LL, LJR, RT, GMDD, and SLH are employees of KalVista Pharmaceuticals.