Efficacy of plasma kallikrein inhibitors in plasma is dependent on the rate of formation of the enzyme-inhibitor complex

Gian Marco De Donatis[1], Nivetha Murugesan[2], Edward J. Duckworth[1], Peter A. Robson[1], Lily Li[2], Louise J. Rushbrooke[1], Edward P. Feener[2] and Sally L. Hampton[1]

KalVista Pharmaceuticals, [1]Salisbury, United Kingdom [2] Cambridge, MA, USA

INTRODUCTION

Plasma kallikrein (PKa) is a serine protease that is derived from its zymogen, plasma prekallikrein (PPK), upon activation of the contact system. The contact system is activated by negatively charged surfaces that activate FXII to FXIIa. FXIIa mediates conversion of PPK to PKa and the subsequent cleavage of high molecular weight kininogen (HK) to generate bradykinin, a potent inflammatory hormone.

C1 inhibitor (C1-INH) is the primary physiological inhibitor of PKa activity. Hereditary angioedema (HAE) is a rare genetic disease caused by C1-INH deficiency, which facilitates PKa hyperactivity leading to episodes of vasogenic edema and pain. C1-INH irreversibly inhibits PKa with a slow association rate.

Selective and potent, orally available small molecule PKa inhibitors have emerged as promising effective and convenient treatments for HAE. Improving our understanding of their mechanisms of action and kinetic parameters could facilitate their optimization as therapies for HAE.

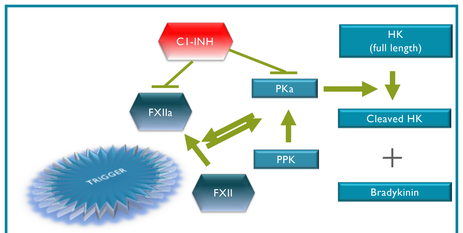


Figure 1: Activation of the contact system. Triggers of attacks in HAE include stress, anxiety, mechanical trauma, infection, hormonal changes, and other factors.

AIM

To identify the biochemical and biophysical properties of PKa inhibitors that contribute to their optimal efficacy in controlling the Kallikrein Kinin System in plasma. These properties are then compared to C1-INH as a therapeutic benchmark for HAE.

METHODS

- Catalytic activity of purified PKa (Calbiochem) was assessed using the fluorogenic substrate (H-D-Pro-Phe-Arg-AFC; Peptide Protein Research). The rate of hydrolysis was monitored by the time dependent increase in fluorescence (IC_{50} data, nM).
- Catalytic activity of PKa in dextran sulfate-activated (DXS, Sigma; 10 µg/ml) plasma (1:4 diluted or neat, VisuCon-F control plasma, Affinity Biologicals Inc) was determined by the time-dependent hydrolysis of fluorogenic substrate. For IC_{50} and efficacy determination, the compounds or C1-INH (Sigma Cat #E0518) were added before (Figures 1-4) or after (Figure 5) the addition of DXS to the plasma.
- The rate of formation of the enzyme-inhibitor complex (K_{on}) was determined using purified PKa rapidly mixed with a solution containing fluorogenic substrate and a concentration range of inhibitor. The time-dependent establishment of inhibition was then used to calculate the rate of formation of the enzyme-inhibitor complex for each concentration of inhibitor. The K_{on} was calculated by plotting the rate of inhibition versus the inhibitor concentration. Data are presented in μM^{-1} sec^{-1}.
- DXS-stimulated cleavage of HK in undiluted plasma was performed in the absence or presence of 300 nM PKa inhibitor and quantified by SDS-PAGE gel electrophoresis, using 7.5% Criterion TGX Precast gels (Biorad). Transfer was made onto Immunobilon-FL PVDF membrane. Image analysis was done using the LICOR imaging system. Mouse monoclonal anti-HK antibody (MAB15692, R&D systems) was used for traditional immunoblotting. Data presented as % of HK remaining after 20 min incubation with DXS compared to HK levels in unstimulated plasma (Table 1).
- Plasma free fraction was determined using "Rapid Equilibrium Dialysis" system (Thermo Scientific), test compounds were prepared at 5 µM in neat human plasma and dialysed against phosphate buffer for 5 hrs at 37°C. Quantification of the compound partitioned in two chambers of the dialysis device was performed via LC-MS/MS. Fraction of compound unbound to plasma proteins presented as % of total.

RESULTS

- In the fluorogenic substrate assay, KVD900 appears as a highly potent inhibitor of PKa with 17-fold and 20-fold potency vs. exogenously added C1-INH in undiluted and diluted plasma, respectively.

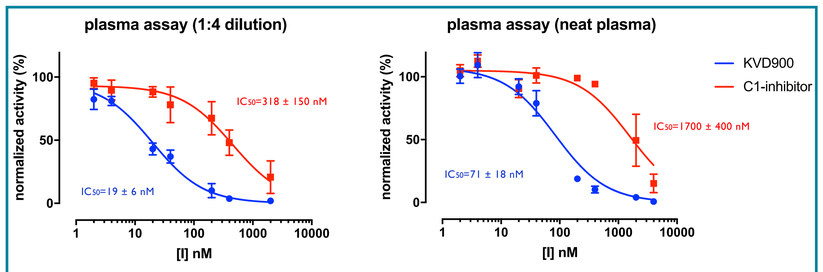
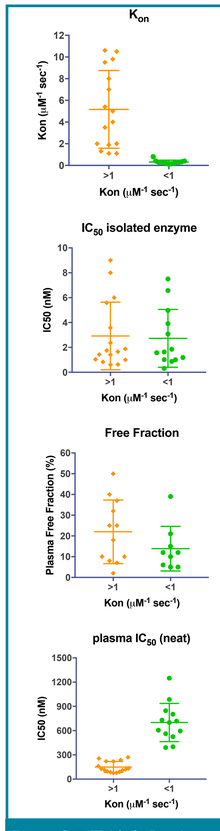


Figure 2: IC_{50} values of KVD900 and C1-inhibitor in dextran sulfate activated human plasma.

- The IC_{50} values of a panel of diverse oral PKa inhibitors ranged from 0.6 to 9 nM for purified PKa and 80 to 700 nM for PKa measured in undiluted plasma (Table 1). The K_{on} for these inhibitors range from 0.1 to ≥10 $\mu M^{-1}sec^{-1}$.



	IC_{50} purified enzyme nM	IC_{50} plasma enzyme nM	K_{on} (μM^{-1} sec^{-1})	Plasma Free Fraction %	HK protection %
C1-INH	50	1700	0.04		
KVD900	6	71	11	25	85
KV998058	0.3	390	0.8		
KV994019	0.6	77	3.5	5	75
KV998074	0.6	161	4.0	25	88
KV998073	0.8	271	2.0	32	83
KV998069	0.9	730	0.2	15	25
KV998051	1.0	221	1.3	7	45
KV998060	1.0	595	0.3		
KV998076	1.0	255	2.7	40	30
KV998068	1.2	525	0.4		
KV994006	1.4	700	0.2	6	7.5
KV998057	1.4	219	1.1		
KV998065	1.4	128	8.0		50
KV998063	1.6	803	0.3	21	
KV998067	1.6	847	0.3	12	
KV998062	1.7	89	11	8	
KV998075	1.8	110	7.0		
KV998061	1.9	562	0.3	5	
KV998072	1.9	86	5.4	10	82
KV998064	2.4	237	1.1	37	10
KV998059	3.1	1249	0.2	10	
KV998055	3.6	95	5.0	10	
KV998071	3.9	400	0.4	39	20
KV998056	5.0	985	0.1	12	
KV998066	5.6	119	1.9		
KV998070	6.6	718	0.3	5	
KV998077	7.5	606	0.6		
KV998052	8.0	133	9.5	50	50
KV998054	9.0	115	9.8	18	84

Figure 3 & Table1: Biochemical profile of the PKa inhibitors analyzed in this study.

- The IC_{50} of PKa inhibitors on purified PKa was a poor indicator of their efficacy on PKa activity (Pearson's $r < 0.3$) or HK cleavage in undiluted plasma (Figure 4). In contrast, a fast K_{on} rate correlates with both a lower IC_{50} for PKa activity in undiluted plasma (Pearson's r ~0.7) and improved protection from DXS-stimulated HK cleavage (Pearson's r ~0.6).

RESULTS

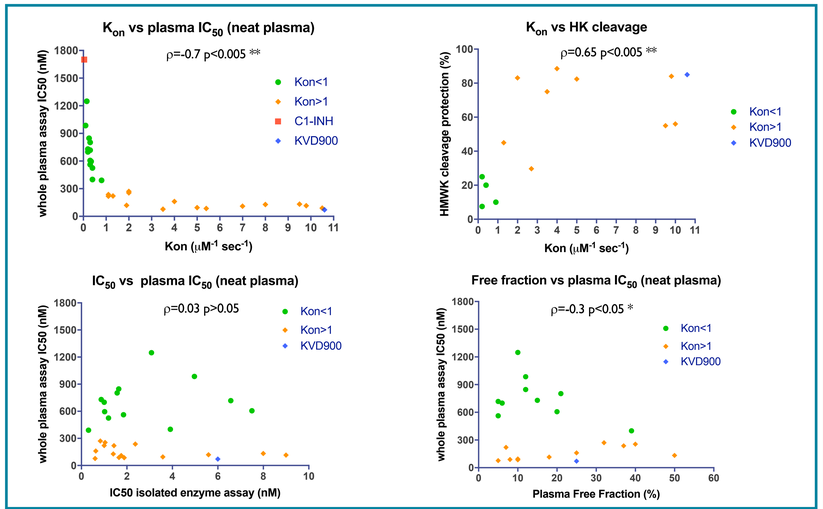


Figure 4:Correlations among K_{on}, IC_{50} (pure PKa), IC_{50} (neat human plasma), plasma protein binding (free fraction) and HK cleavage for PKa inhibitors listed in Table 1. PKa inhibitors are displayed with K_{on} <1 (green), K_{on} >1 (orange), and KVD900 (blue). Pearson correlations are shown.

- From these analyses we identified a novel and potent PKa inhibitor, KVD900, with fast kinetic parameters (K_{on}>10 $\mu M^{-1}sec^{-1}$), that was highly effective in reducing the PKa activity and HK cleavage in undiluted plasma. The effect of fast K_{on} as a property for rapid inhibition was compared in plasma by observing inhibitory potential by addition to the plasma reaction after it had been initiated.

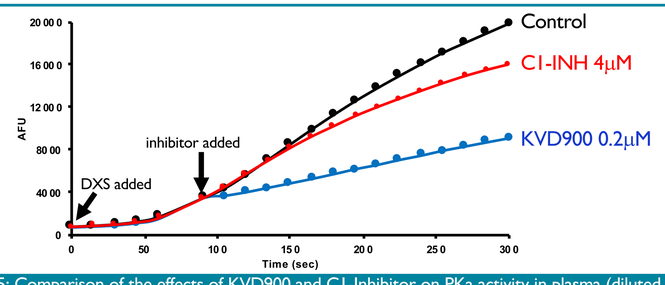


Figure 5: Comparison of the effects of KVD900 and C1-Inhibitor on PKa activity in plasma (diluted 1:4) activated with dextran sulfate (DXS). Inhibitors, at concentration 10X their IC_{50}, were added to plasma approximately 100 seconds after the addition of DXS.

SUMMARY & CONCLUSION

- The PKa inhibitor IC_{50} on pure enzyme is not an adequate predictor of PKa IC_{50} in undiluted plasma or efficacy in protecting HK from DXS-stimulated HK cleavage.
- Inhibitors with similar potency for pure PKa can differ markedly in K_{on} kinetics. Our findings suggest that the rate constant for the entrance of the inhibitor into the active site of the enzyme (K_{on}) influences inhibitor efficacy in plasma.
- Inhibitors with higher K_{on} values ~10 $\mu M^{-1}sec^{-1}$ displayed proportionally lower IC_{50} in the plasma assay and more protection against HK cleavage, compared with inhibitors with slower K_{on} rates.
- Discriminating drug candidates for their K_{on} could be a useful consideration in the optimization PKa inhibitors as treatments for HAE.

CONFLICT OF INTEREST DISCLOSURE

- GMDD, NIM, EJD, PAR, LL, LJR, EPF, SLH are employees of KalVista Pharmaceuticals.