3144

Factor XIIa inhibition protects against VEGF-induced neuroretinal dysfunction in mice.

Allen Clermont, Nivetha Murugesan, Edward P. Feener

KalVista Pharmaceuticals: Cambridge, MA USA.

INTRODUCTION

- The kallikrein-kinin system (KKS) has been implicated in contributing to both VEGF-independent and -dependent mechanisms of diabetic macular edema (DME)[1-3].
- Previous studies have demonstrated that activation of the KKS increases retinal thickness and causes vascular dysfunction [4-6], however, little is known about the effects of the KKS on visual function.
- Factor XIIa (FXIIa), the primary activator of the KKS, has been identified in the vitreous of patients with DME [2-3].
- Previously we have shown that intravitreal injection of VEGF or bradykinin (BK) induced alterations in electroretinogram (ERG) amplitude in rats and that these effects are mediated through bradykinin B2R[7].
- Here we further examine the effects of the KKS on neuroretinal function.

AIM

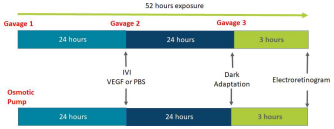
This study evaluates the effects of the novel oral FXIIa inhibitor KV998086 on neuroretinal dysfunction induced by VEGF in mice.

METHODS

Scotopic ERG: Retinal neuronal function was assessed using full field dark-adapted scotopic ERG (Celeris, Diagnosys, Andover, MA). C57bl/6 mice were dark adapted for 3 hours prior to stimulation with a 10 cd-s/m^2 flash intensity. Electrodes were placed in the touch-touch configuration. 3 sweeps were averaged per eye at 2 minute intervals between sweeps. Data was collected and analyzed using the Espion V6 (V6.64.14) software and Excel.

Intravitreal injection (IVI): Eyes of male C57bl/6 or FXII$^{-/-}$ were dilated with 1% tropicamide (Somerset Therapeutics, FL). Single infusions were made using a 10µL Gastight syringe (model 1701RN) with a 32-gauge needle intravitreal injections (1µL/eye) of recombinant vascular endothelial growth factor (VEGF-165) (100ng/eye, #PHC9394, Life Tech, Carlsbad, CA), human Factor XIIa (FXIIa) (50ng/eye, Enzyme Research Labs, South Bend, IN), Bradykinin (BK) (20µM, Bachem, Vista, CA) or Dulbecco's phosphate buffered saline (PBS) (D8537, Millipore-Sigma, Burlington, MA) control. ERG were measured at 24 or 48 hours post IVI.

FXIIa inhibition studies: Systemic inhibition of FXIIa was initiated by subcutaneous (SC) or oral (PO) administration of the FXIIa inhibitor, KV998086. For SC studies, mice were implanted with an osmotic pump (Alzet 1003D) containing KV998086 (infusion rate 14.2mg/kg/d) initiated at 24 hours prior to IVI. For oral studies, mice received KV998086 at 45 mg/kg as shown below.



Optokinetics: Visual spatial frequency thresholds were measured with an opto-kinetic tracking system (OptoMotry System, Cerebral Mechanics, Lethbridge, Canada). Maximum spatial frequency at a fixed contrast was measured per mouse eye at baseline and at 6, 12, 24 hours and 7 days post IVI of BK (20µM) or PBS in the contralateral eye.

Animals: Wildtype (WT) C57bl/6 mice were purchased from JAX labs. FXII$^{-/-}$ mice were backcrossed on C57bl/6 background for 9 generations.

RESULTS

Figure 1: Time course of IVI BK on optokinetic response in mice

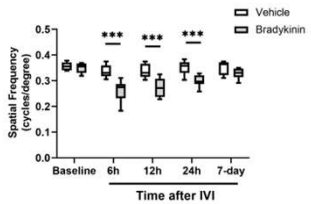


Figure 1: Spatial frequency response to IVI BK or PBS measured by optokinetics (n=9 mice/group) in WT mice. Measurements were obtained at baseline and at 6h, 12h, 24h and 7 days after IVI of 1 µL BK (2µM final) or PBS. Decreases in spatial frequency responses are observed between 6 to 24 hours post IVI of BK. ***p<0.001

Figure 2: Effect of IVI Bradykinin on scotopic ERG

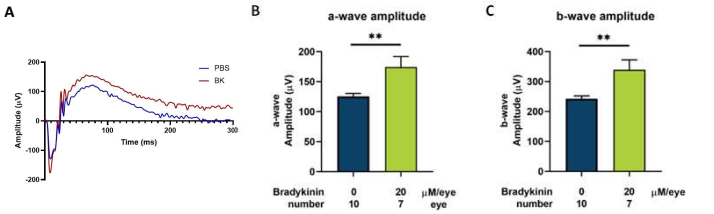


Figure 2: (**A**) Scotopic ERG traces from eyes following BK or PBS injection. The ERG responses from eyes receiving an intravitreal injection of BK displayed increased amplitude for (**B**) a-wave by 38% (**p<0.01) and C) b-wave by 53% (**p<0.01) compared to PBS injection at 24 hours post injection.

Figure 3: Effect of IVI FXIIa on scotopic ERG

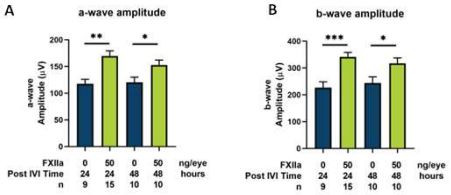


Figure 3: Scotopic ERG responses from eyes at 24 hours and 48 hours after receiving an IVI of FXIIa or PBS are shown. At 24 hours post injection, FXIIa increased the signal amplitude for a-wave (**A**) by 44% (**p=0.001), b-wave (**B**) by 51% (***p<0.001) compared to PBS injection. At 48 hours post injection, FXIIa increased the signal amplitude for a-wave by 28% (*p=0.027) and b-wave by 30% (*p=0.029) compared to PBS. Data demonstrates that KKS activation causes ERG amplitude effects as observed by BK.

Figure 4: Effects of IVI VEGF on scotopic ERG in WT and FXII$^{-/-}$ mice

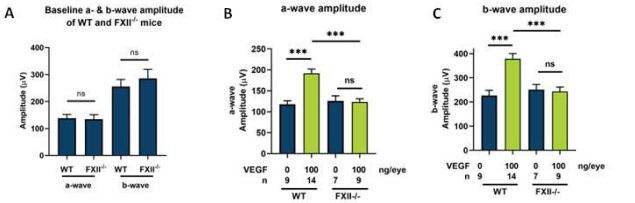


Figure 4: : The effects of VEGF and PBS on scotopic ERG traces were obtained from WT and FXII$^{-/-}$ mice at 24 hours post IVI. (**A**) Baseline ERG amplitudes were similar in both WT and FXII$^{-/-}$ mice. At 24 hours post injection, VEGF increased (**B**) a-wave amplitude by 63% (***p<0.001) and (**C**) b-wave by 71% (***p<0.001) compared to PBS injection in WT mice. VEGF did not significantly affect amplitudes of the a-wave (ns) and b-wave (ns) compared to PBS injection in FXII$^{-/-}$ mice. Data demonstrates that FXII$^{-/-}$ mice are protected against VEGF-induced scotopic ERG effects. This suggests that VEGF effects upon ERG amplitude are mediated through the KKS.

Table 1. Potency and selectivity of the oral FXIIa inhibitor KV998086

	KV998086
Enzyme	IC_{50}
Factor XIIa	7.2 nM
Factor XIa	>40 µM
Factor Xa	>40 µM
Factor VIIa	>10 µM
Plasma Kallikrein	>40 µM
Plasmin	>40 µM
Thrombin	16 µM
Trypsin	>40 µM
Tissue Kallikrein 1	7.9 µM

Table 1: KV998086 protease selectivity[8]
IC_{50} (half maximal inhibitory concentration) values for KV998086 for human Factor XIIa and a panel of other serine proteases in isolated enzyme kinetic substrate cleavage assays. The mouse FXIIa IC_{50} is 9.4nM.

Figure 5: Effect of KV998086 on VEGF-induced scotopic ERG amplitude

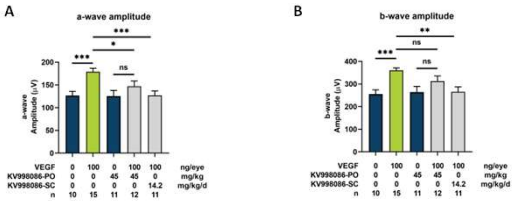


Figure 5: Effect of systemically delivered FXIIa inhibitor KV998086 on VEGF-induced scotopic ERG amplitudes in WT mice at 24 hours. The VEGF-induced increase in amplitude was reduced in mice pretreated with an oral administration of KV998086 (PO) by 61.3% for a-wave (*p<0.05) (**A**) and 46% for b-wave (ns) (**B**). Minipump administration of KV998086 (SC) reduced VEGF induced amplitude increase by 99% for a-wave (***p<0.001) (**A**) and 90% for b-wave (**p<0.01) (**B**). These data demonstrate that systemic delivery of the oral FXIIa inhibitor KV998086 protects against VEGF induced scotopic ERG amplitude effects as observed in FXII$^{-/-}$ mice.

SUMMARY & CONCLUSIONS

- Intravitreal injection of BK decreased opto-kinetic spatial frequency and increased ERG amplitude
- FXIIa and VEGF caused abnormalities in scotopic ERG amplitude comparable with those caused by BK.
- Systemic administration of the oral FXIIa inhibitor KV998086 and FXII knockout (FXII$^{-/-}$) blocked the effects of VEGF on ERG.
- These data suggest that systemic administration of an oral FXIIa inhibitor may provide protective effects on neuroretinal function in DME.

DISCLOSURE

ACC, NM and EPF are employees of KalVista Pharmaceuticals.

REFERENCES

1. Clermont et al., **Plasma kallikrein mediates vascular endothelial growth factor-induced retinal dysfunction and thickening** *Invest Ophthalmol Vis Sci*. 2016 May 1;57(6):2390-9
2. Gao et al., 2007 **Extracellular carbonic anhydrase mediates hemorrhagic retinal and cerebral vascular permeability through prekallikrein activation** *Nat Med*. 2007 Feb;13(2):181-8.
3. Kita et al., 2015 **Plasma kallikrein-kinin system as a VEGF-independent mediator of diabetic macular edema** *Diabetes*. 2015 Oct;64(10):3588-99
4. Phipps et al., **Plasma kallikrein mediates angiotensin II type 1 receptor-stimulated retinal vascular permeability** *Hypertension*. 2009 Feb;53(2):175-81
5. Clermont et al., **Plasma kallikrein mediates retinal vascular dysfunction and induces retinal thickening in diabetic rats** *Diabetes*. 2011 May;60(5):1590-8
6. Liu et al., **Intraocular hemorrhage causes retinal vascular dysfunction via plasma kallikrein** *Invest Ophthalmol Vis Sci*. 2013 Feb 7;54(2):1086-94
7. Clermont et.al. **Effects of vascular endothelial growth factor (VEGF) on electroretinogram (ERG) amplitude in rats are mediated by bradykinin.** Annual Meeting of the Association for Research in Vision and Ophthalmology 2017 58(8): 53472017 58(8): 5347
8. Clermont, et.al. **Oral FXIIa inhibitor KV998086 suppresses FXIIa and single chain FXII mediated kallikrein kinin system activation** *Front Pharmacol*, 2023, Dec, 19:14:1287487

CONTACT INFORMATION

Allen Clermont,
Director, In Vivo Pharmacology,
KalVista Pharmaceuticals.
Allen.Clermont@kalvista.com

Slide 1

EPF0 Were both p values exactly 0.01 or < 0.01?
Edward P Feener, 2024-05-01T20:41:21.672

EPF1 Adjusted box for the intro to center the text
Edward P Feener, 2024-05-01T20:42:33.495