Novel oral plasma kallikrein (PKa) inhibitors KV998052 and KV998054 ameliorate VEGF-induced retinal thickening in a murine model of retinal edema

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INTRODUCTION

Plasma kallikrein (PKa) has been implicated in mediating both VEGF-independent and VEGF-dependent pathways of diabetic macular edema (DME)1-3. PKa is a serine protease that is derived from plasma prekallikrein (PPK), an abundant circulating zymogen. PKa cleaves high molecular weight kinogen (HK) to generate bradykinin, which is increases vascular permeability and inflammation. Previous studies have shown that PKa and HK are increased in vitreous samples from people with DME 1,2. Here we investigate the effects of two novel, structurally diverse and orally available PKa inhibitors, KV998052 and KV998054, on the prevention and reversal of VEGF-induced retinal edema in mice.

AIM

To evaluate the effects of two novel, orally-available PKa inhibitors, KV998052 and KV998054, on the protection of PKa-mediated HK cleavage in mouse plasma in an ex vivo assay and test their pharmacodynamic effects in vivo in a mouse model of VEGF-induced retinal edema.

METHODS

PKa enzyme activity:

Effects of KV998052 and KV998054 on PKa catalytic activity were assayed using H-D-Pro-Phe-Arg-AFC. Inhibitor selectivity was assessed using a panel of closely-related serine proteases. Effects on HK cleavage were measured in whole plasma stimulated with dextran sulfate (DXS) using a capillary-based immunoassay.

Ex vivo whole plasma HK cleavage assay:

HK cleavage in undiluted citrated C57BL/6 mouse plasma was stimulated by contact system activation with DXS (Sigma #31395-10G; 6.25 µg/ml) on wet ice. Working stock solutions of KV998052 and KV998054 in DMSO were prepared and diluted in phosphate buffered saline (PBS) and used at the respective final concentrations described.

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

Preparation of samples: Plasma samples were diluted 1:200, combined with fluorescent master mix, vortexed, heated at 95°C for 5 minutes, and loaded onto the WES plate. Goat anti-mouse HK and plasma pre-kallikrein/kallikrein (PPK/PKa) antibodies were used for this chemiluminescence-based detection method using the WES System (ProteinSimple). Gray scale images corresponding to peaks immunoreactive HK, PPK, and PKa were visualized by chemiluminescence using Compass for SW software.

Pharmacodynamics:

Retinal thickening was measured in a mouse model of VEGF-induced retinal edema with KV998052 or KV998054 administered s.c. at doses up to 2mg/kg/day using Alzet pumps or orally 50mg/kg bid. Mice receiving equivalent volumes of corresponding vehicles were used as controls. Plasma concentrations were determined by LC-MS/MS. Retinal thickness was measured with optical coherence tomography (OCT), at baseline and time points post intravitreal injections of VEGF (100ng/eye) or PBS vehicle in both prevention and intervention studies using Bioptigen Diver 3.3.7.

RESULTS

Table 1: Potency & selectivity of KV998052 & KV998054

<table>
<thead>
<tr>
<th>Target</th>
<th>KV998052 (3nM)</th>
<th>KV998054 (4nM)</th>
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<tbody>
<tr>
<td>PKa (Human/Mouse)</td>
<td>11.1nM</td>
<td>11.4nM</td>
</tr>
<tr>
<td>Tissue Kallikrein</td>
<td>&gt;6000</td>
<td>&gt;6000</td>
</tr>
<tr>
<td>Factor XII</td>
<td>&gt;6000</td>
<td>&gt;6000</td>
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<tr>
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<td>Plasma</td>
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<tr>
<td>Plasmin</td>
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<tr>
<td>Thrombin</td>
<td>&gt;6000</td>
<td>&gt;6000</td>
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<tr>
<td>Trypsin</td>
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PKa activation and HK cleavage: scheme

Figure 1: Dose response of KV998052 and KV998054 on DXS-stimulated HK and PPK cleavage in undiluted mouse plasma.

Figure 2: Systemically administered KV998052 and KV998054 inhibits VEGF induced retinal thickening in mice. Pharmacodynamic effects correlate with both plasma and retinal concentrations of PKa inhibitor.

Figure 3: Oral administration of KV998054 (50mg/kg) prevents VEGF induced retinal edema at 24h post intravitreal (IVT) injection.

Figure 4: Oral administration of KV998052 (10mg/kg) after onset of retinal edema accelerated the resolution of VEGF induced retinal thickening in mice. Thickening of the inner nuclear layer (INL) was reversed at 72h.

SUMMARY & CONCLUSION

• KV998052 and KV998054 are potent, selective and orally-available PKa inhibitors. Both provide dose dependent protection against PKa generation and HK cleavage ex vivo in DXS stimulated mouse plasma.

• Infusion of KV998054 at 1 and 2 mg/kg/day s.c. reduced VEGF-stimulated edema at 24h by 40%, p<0.0026 and 54%, p=0.001, respectively.

• Oral administration of KV998054 initiated before VEGF injection resulted in a 59% (p<0.001) decrease in VEGF retinal thickening at 24h.

• Oral KV998052 provided 24h after intravitreal injection of VEGF accelerated resolution of edema at 72h by 83% (p=0.015) compared with controls receiving vehicle.

• Retinal segment analysis revealed that reduction in thickening by PKa inhibition occurred in multiple layers, including an 87% decrease in the inner nuclear layer.

• In summary, oral PKa inhibitors have been identified that prevent and reverse VEGF-induced retinal edema.

CONFLICT OF INTEREST DISCLOSURE

NM, SJF, LL, EJD, SLH and EPF are employees of KalVista Pharmaceuticals. Work done by ACC was funded by KalVista Pharmaceuticals.