Novel topical antiangiogenic SRPK1 inhibitors in the treatment of age-related macular degeneration

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INTRODUCTION

Age-related macular degeneration (AMD) is a disease of the central macula and the leading cause of blindness in people over 50 years of age. Exudative (wet) AMD is characterised by choroidal neovascularization (CNV), the leakage of blood vessels and serous fluid from the neovasculature beneath the retinal pigment epithelium (RPE), leading to the loss of photoreceptors, retinal detachment, macular scarring and visual loss. Current treatment regimes for AMD involve costly monthly intraocular injections, with non-specific and weakly potent humanized anti-vascular endothelial growth factor (VEGF) antibody therapies. Serine-rich protein kinase-1 (SRPK1) has been identified as a regulator of pro-angiogenic VEGF splicing by phosphorylating serine-rich splicing factor-1 (SRSF1), which binds to VEGF pre-mRNA. A novel SR phosphorylation Inhibitor (SPHINX) compound that inhibits SRPK in the micromolar range and regulates VEGF gene splicing has been recently developed[1].

AIM

To test the efficacy of SRPK inhibitors e.g. SPHINX and its derivatives, as anti-angiogenics and to identify good candidates for therapeutic use in AMD and other diseases.

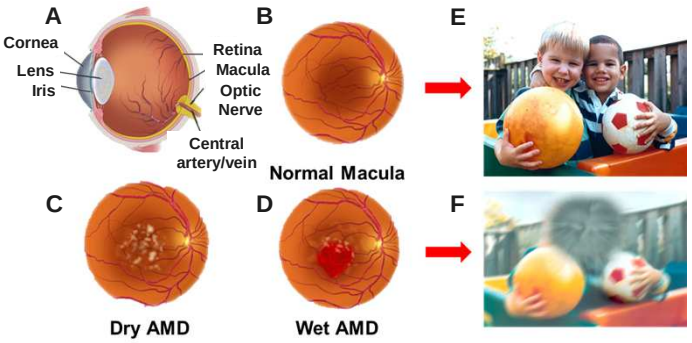

Figure 1 (A) Anatomy of the eye. **(B)** The macula lutea is an oval shaped and highly pigmented area in the centre of the retina, with a diameter of approximately 6mm. At its centre is the fovea, a small pit that contains the largest concentration of cone cells and is responsible for central, high resolution vision. **(C)** A fundus photograph depicting characteristic drusen deposits that accumulate between the retina and the choroid as seen in dry AMD. **(D)** A fundus photograph showing wet AMD, with blood vessel growth up from the choroid behind the retina. **(E)** A photograph as seen by an individual with normal vision. **(F)** A photograph as seen by a patient with advanced AMD.

METHODOLOGY

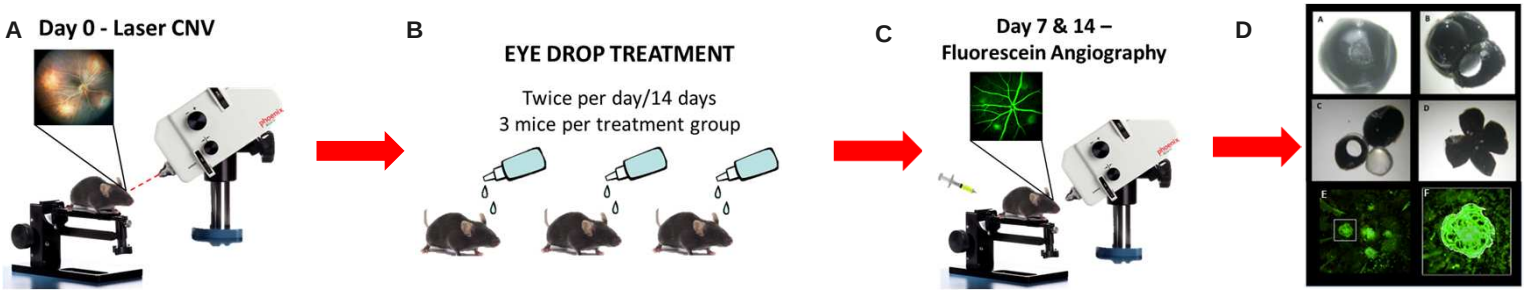

Figure 2 (A) Laser-induced CNV Model. Four laser spots (Meridian 532ª green laser, 250 mW, 100 ms, Iridex, CA) were delivered to each eye in 6-8 week old C57/B6 mice, avoiding major vessels. Rupture of the Bruch's membrane was confirmed by a subretinal bubble. **(B) Treatment.** Immediately following laser photocoagulation the mice received topical eye drops of either SRPIN340 (positive control, 10µg/ml), SPHINX7 (10µg/ml), or SPHINX31 (0.2, 2, or 20µg/ml) twice daily (10µl) for 14 days. The left eye received vehicle only and the right eye received SPHINX treatment. **(C) Fluorescein Angiography.** Mice were given an intraperitoneal injection of fluorescein and angiograms of the laser induced lesions in both eyes were imaged at day 7 and day 14. The area of each lesion was measured using ImageJ software and the percentage area was calculated based on the vehicle only control lesion areas. **(D) Sclera choroid flat mount preparations and Isolectin-B4 histology.** Eyes were fixed with 4% PFA for 30 mins and the anterior components of the eye were dissected out, the retina and hyaloid vessels were removed and four radial incisions were made to flat mount the scleral choroid cup. The tissue was blocked with 1 x PBS/0.05% Tween 20 prior to staining with isolectin B4 (2µg/ml) overnight at 4ºC, which is a marker of endothelial cells lining blood vessels.

RESULTS

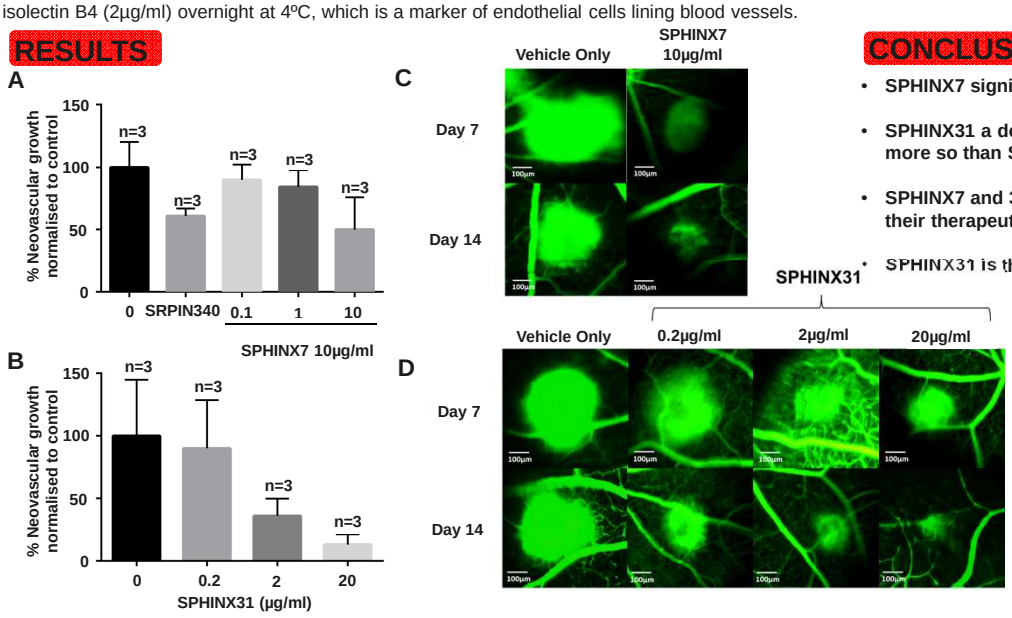

Figure 3. SPHINX compounds inhibit laser induced choroidal neovascularisation following administration of topical eye drops. 3 C57/Bl6 mice per group were subjected to four laser lesions to the back of the eye on day 0. From day 1 to 14 mice received twice daily topical treatments of **(A)** SPHINX7 or SRPIN340, or **(B)** SPHINX31 at concentrations shown. Lesions were imaged by fluorescein angiography **(C and D)**. Dose response to SPHINX31. IC50 =1.84µM (~1µg/ml) **(E)**.

CONCLUSIONS

- **SPHINX7 significantly reduced lesion size in a mouse model of CNV.**
- **SPHINX31 a derivative of SPHINX7 significantly reduced lesions size and more so than SPHINX7.**
- **SPHINX7 and 31 penetrate the anterior of the eye to the posterior to exert their therapeutic action.**
- **SPHINX31 is therapeutically more potent than SPHINX7.**

REFERENCES

[1] Nowak (2010). J Biol Chem 285:3487-95.

[2] Gammons (2013). IOVS 54:6052-662

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