A novel approach in preventing vascular leakage and angiogenesis in wet age-related macular degeneration

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**Vascular leakage and angiogenesis in diseases:** The vascular system carries blood throughout the body to supply oxygen and nutrients to tissues and remove waste from tissues. Dysfunction of the vascular system worsens many diseases. In this perspective, vascular function in blinding eye diseases, current therapies, and our developing approach will be introduced.

The blood vessels are composed of the monolayer of the endothelial cells, and the outer layer made by pericytes and smooth muscle cells provide structural support and shape to the vessel. Endothelial monolayers function as a barrier. The barrier function of endothelial monolayers controls plasma protein uptake and the infiltration of immune cells by regulating adherence of cell-cell junction. Vascular leakage causes unwanted plasma leak, accumulation of immune cells, and tissue inflammation.

Angiogenesis is a new formation of the blood vessels from pre-existing vessels. To form the new sprouting from the vessels, the endothelial monolayer first loosens their cell-cell junction, thus the endothelial permeability is the first step of angiogenesis. Newly formed vessels do not have the outer layer yet. Therefore, vascular leakage is often associated with angiogenesis.

Vascular leakage and angiogenesis are pathogenesis in many diseases such as cancer metastasis, wet age-related macular degeneration (AMD), and diabetic retinopathy. Among many factors regulating vascular leakage and angiogenesis, vascular endothelial growth factor (VEGF) is a critical factor. Thus, targeting VEGF pathway is an important promising approach for these diseases.

**Current therapies for blinding eye diseases:** Macular degeneration is an eye disease causing a loss in the center of the field of vision. Most of the patients (90%) with AMD have “dry” AMD. In dry AMD, the macula layers get progressively thinner and functioning less, which is called atrophy. Dry AMD also shows pigment discoloration and tissue inflammation.

Vascular leakage is often used (Ehlken et al., 2014). Moreover, switching the therapy to other therapies often improves the efficacy to inhibit angiogenesis and vascular leakage in wet AMD and improved the vision or delayed the disease progression. However, repeating monthly injections is a big burden for old patients and reduces responsiveness of patients to the same therapy. In fact, switching the therapy to other therapies with different mechanisms of action is often used (Ehlken et al., 2014). Moreover, some patients (~20%) do not respond to anti-VEGF therapies (Schauwvlieghe et al., 2016). Therefore, novel strategies targeting the VEGF/VEGFR2 pathway with different mechanisms constitute a fertile opportunity for drug development.

**A novel approach for blinding eye diseases:** VEGF induces angiogenesis and vascular leakage by binding and activating its receptor on the cell surface, VEGFR2 (Simons et al., 2016). Upon binding with VEGF, VEGFR2 is internalized and dynamically trafficked between intracellular membrane compartment and cell surface while activating downstream signaling from each location (Simons et al., 2016). As part of activated VEGFR2 undergoes to degradation pathway, more synthesized VEGFR2 is transported to the cell surface by a kinesin family protein, KIF13B (Yamada et al., 2014). To regulate the degree of activation of VEGF/VEGFR2 signaling, the available amount of VEGFR2 on the cell surface is one of the critical regulating mechanisms. Therefore, inhibition of KIF13B critically reduces VEGF-induced events, such as endothelial cells migration, sprouting, and angiogenesis (Yamada et al., 2014). We took advantage of the finding to develop the novel inhibitor of VEGF/VEGFR2 signaling. Based on the binding site between KIF13B and VEGFR2, we designed a 23 aa peptide (3.0 kDa), kinesin-derived angiogenesis inhibitor, which competes with the binding between endogenous KIF13B and VEGFR2, thus inhibits KIF13B-mediated VEGFR2 trafficking to the cell surface (Figure 1) (Yamada et al., 2017). Interestingly, KAI treatments significantly inhibited tumor angiogenesis and tumor growth in subcutaneous xenograft model of human lung carcinoma (Yamada et al., 2017).
The role of KIF13B in tumor angiogenesis and tumor growth was also confirmed by genetic deletion of KIF13B in EC in mice (under review). Although KIF13B has other functions to transport other molecules in the cells (Horiguchi et al., 2006; Yamada et al., 2007; Tong et al., 2010; Noseda et al., 2016), KAI was designed to selectively inhibit the interaction of VEGFR2 with KIF13B, by not overlapping with other binding sites for other molecules (Yamada et al., 2017). Thus, the effect of KAI is most likely not related to other functions of KIF13B and is selective inhibition of trafficking of VEGFR2 to the cell surface.

Similar to bevacizumab, we applied our strategy to wet AMD using a mouse model, laser-induced choroidal neovascularization. KAI treatment was as effective as current therapy anti-VEGF to reduce laser-induced choroidal neovascularization (Waters et al., 2021). The advantage of using KAI is its size, which enables its use as an eyedrop. Moreover, KAI is designed to be able to penetrate through the cell membrane using cationic residues. The strategy is similar to other cationic cell-penetrating peptides such as antennapedia (Reissmann and Filatova, 2021). KAI successfully penetrated the cells without showing any toxicity, i.e., no effect on proliferation or survival (Yamada et al., 2017). Topically applied KAI as an eyedrop successfully reaches the back of the mouse eyes (Waters et al., 2021). Daily treatment of KAI inhibited neovascularization in the laser-induced choroidal neovascularization model compared with the control peptide (Waters et al., 2021). Together, these data suggest the potential benefit of developing KAI eyedrop for future clinical use for non-responders of anti-VEGF therapies.

Comparing current therapy and KAI, and potential combination approach: VEGF/VEGFR2 pathway is involved in many diseases. Increased local VEGF plays a central causative role in wet AMD, diabetic retinopathy, diabetic macular edema, neovascular glaucoma (Apte et al., 2019). Excessive VEGF to these diseases induces vascular leakage, angiogenesis, and inflammation, damaging the tissue (Apte et al., 2019). Therefore, the therapeutic effect of the anti-VEGF approach is expected in these diseases, and confirmed by mouse models, and approved for clinical use by the FDA (Apte et al., 2019). We showed the promising efficacy of KAI for the mouse model of wet AMD (Waters et al., 2021). It is most likely KAI is also efficient for these eye diseases related to VEGF. As VEGF is also important in development, the use of anti-VEGF for babies and children needs some caution, i.e. effect on brain development, etc. Therefore, careful investigation in animal models will be needed for testing efficacy, toxicity, and any adverse effect.

We showed topically applied KAI from the top of the eye reaches to the back of the eye by immunostaining and quantification by competitive enzyme-linked immunosorbent assay (Waters et al., 2021). Topically applied drugs from the top of the eye have several drug entry pathways, such as corneal, conjunctival, and scleral pathways (Mandal et al., 2018). Although the absorption route of topically applied KAI is unknown, we expect KAI in circulation can enter EC regardless of the blood-retina barrier. KAI enters cultured retina EC in vitro, and KAI applied by eyedrop was detected in the choroidal vessels in mouse eyes (Waters et al., 2021). The absorption rate and metabolism of KAI in the eyes are under investigation. To further test biodistribution and pharmacokinetics of KAI, examination in bigger animals such as rabbits, pigs, or monkeys would be needed. Nonetheless, development of a new topically applicable drug is beneficial for non-responders to current anti-VEGF therapies, the patients who lose responsiveness after repeated treatments, and for the patients who have difficulty visiting the doctor’s office monthly. KAI is also promising to be used as a combination or adjuvant therapy with current therapies for eye diseases.

Conclusion: Current therapies for wet AMD are effective in improving vision or delaying disease progression for most patients. However, developing eyedrop as a therapy for wet AMD will provide more options for patients, reduces non-responsiveness, and prevent blindness for more patients.

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