SZN-413, a FZD4-specific Agonist, as a Potential Therapeutic for the Treatment of Diabetic Retinopathy

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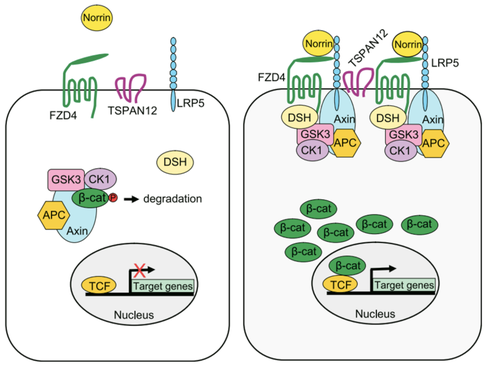
Background

Wnt signaling is one of the central mechanisms regulating tissue morphogenesis during embryogenesis and maintaining tissue homeostasis and repair in adults.[1-5] In retinal endothelial cells, Wnt/β-catenin signaling is mediated by the Norrin-FZD4LRP5-TSPAN12 signaling complex (Figure 1). This signaling pathway is fundamental for the development of retinal vasculature as mutations in genes involved in this signaling complex—NDP, FZD4, LRP5, and TSPAN12—cause familial exudative vitreoretinopathy.[6-8] Within this signaling complex, FZD4-mediated Wnt signaling is essential to maintain retinal vascular integrity during postnatal tissue homeostasis.[9-10] Activation of FZD4-mediated signaling induces gene expressions of tight junction proteins such as claudin-5 (Cldn5), zonula occludens-1 (Zo1), major facilitator superfamily domain containing 2 (Mfsd2) for vascular barrier functions and reduces the expression of plasmalemma vesicle associated protein (PLVAP), a marker of immature or leaky retinal vessels.[10-12]

Diabetic retinopathy (DR), characterized by loss of normal retinal vasculature and areas of retinal non-perfusion, may lead to vision loss through the development of diabetic macular edema (DME) and proliferative DR.[13] Anti-VEGF agents are the current standard of care for DME and DR as they control ocular neovascularization, leakage, and intraocular inflammation, but they do not induce reperfusion of ischemic retinal areas.[13]

Figure 1. Overview of Norrin/β-catenin signal transduction



In retinal endothelial cells, Norrin is the ligand for FZD4 and signal transduction is mediated by the Norrin-FZD4-LRP5-TSPAN12 signaling complex[14], resulting in the translocation of β-catenin into the nucleus and subsequent activation of Wnt target genes.

Purpose

These studies examined whether a novel Norrin mimetic could promote the regeneration of damaged blood vessels and their functions in diabetic retinopathy animal models.

Methods

A novel, bi-specific Norrin mimetic (SZN-413-p, Figure 2) targeting FZD4 and LRP5 was examined for its efficacy in animal models of retinopathy. In a mouse model of oxygen-induced retinopathy (OIR), avascular (AV) and neovascular (NV) areas were measured 5 days post-treatment from retinal flat mount images. Furthermore, impact on vascular leakage by SZN-413 was examined in a rabbit model of VEGF-induced retinal vascular leakage; the level of fluorescein leakage was scored in a blinded manner 3 days post-damage induction. ANOVA was used for statistical analysis.

Results

In the mouse OIR model (Figure 3A), nanogram quantities of SZN-413-p and SZN-413 significantly reduced NV area size ($p<0.001$) to a level comparable to that of the positive control (aflibercept) group (Figure 3B, C). Both SZN-413-p and SZN-413 showed a significant reduction in AV area size compared to that of vehicle ($p<0.001$) and aflibercept groups ($p<0.05$, $p<0.01$ for SZN-413-p, SZN-413 groups, respectively) (Figure 3C). In the rabbit model of VEGF-induced retinal vascular leakage, SZN-413 significantly reduced retinal vascular leakage by ~80%, compared to that of the vehicle-treated group ($p<0.01$) (Figure 4).

Figure 2. SZN-413-p is a FZD4-specific agonist

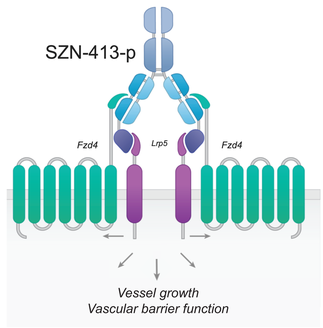
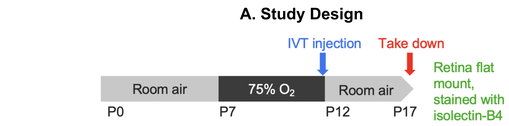
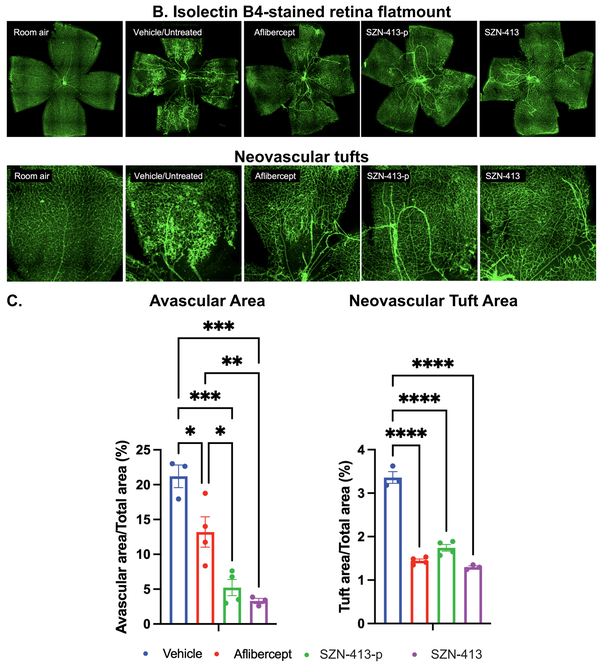


Figure 3. SZN-413-p and SZN-413 were efficacious in a mouse model of oxygen-induced retinopathy

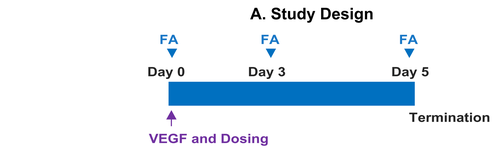


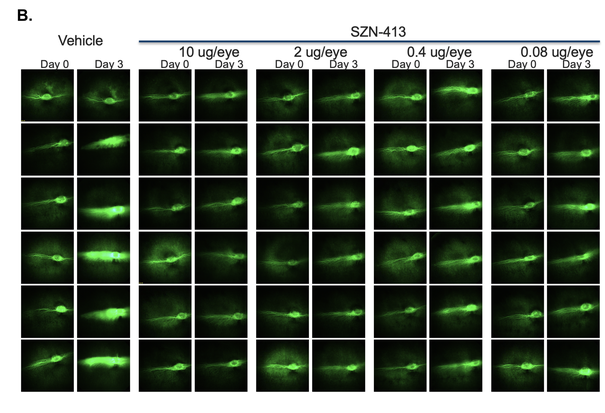
Treatment
Vehicle (HBS)
Aflibercept, 60 µg/1 µL/eye (260 µM)
SZN-413-p, 68 ng/1 µL/eye (200 nM)
SZN-413, 13.6 ng/1 µL/eye (40 nM)

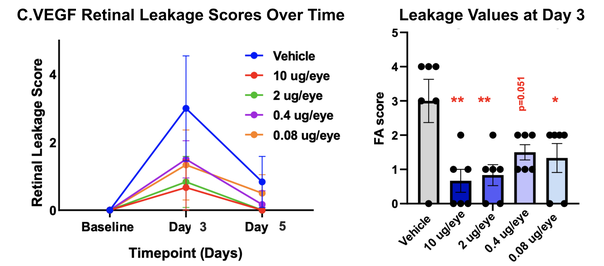


(A) Timeline of the OIR mouse model. Vehicle, SZN-413-p, SZN-413, and aflibercept were administered by intravitreal injection at P12, and all mice were euthanized at P17 for retinal flat mounts. (B) Representative retinal flat mount images. Bottom panels show the pathologic NV tufts. (C) Results of morphometric analysis of retinal flat mounts measuring area of NV tufts and AV areas. Values are mean ± SEM, *p <0.05, **p <0.01, ***p <0.001.

Figure 4. SZN-413 was efficacious in a rabbit model of VEGF-induced retinal vascular leakage







(A) Timeline of the VEGF-induced retinal vascular leakage rabbit model. Vehicle or 4 dose levels of SZN-413 were administered by intravitreal injection at day 0. Retinal leakages were monitored at days 0, 3, and 5 by fluorescein angiography (FA). (B) Representative FA images from each group of indicated treatments. (C) Trends of retinal leakage score changes in each group. The leak levels peaked on day 3, after which they regressed on day 5 in all groups. Graphs representing the FA scores of each group at day 3. Values are mean ± SEM, *p <0.05, **p <0.01.

References: 1. Junge, et al., 2009. 2. Paes, et al., 2011. 3. Y. Wang, et al., 2012. 4. Ye, et al., 2009. 5. Diaz-Coranguez, et al, 2020. 6. Gariano & Gardner, 2005. 7. Kondo, et al., 2013. 8. Yang, et al., 2012. 9. Diaz-Coranguez, et al, 2020. 10. Y. Wang et al., 2018. 11. Chidiac, et al., 2021. 12. Z. Wang, et al., 2020. 13. Wykoff, et al., 2022. 14. Panagitou et al., 2017.

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Summary

- **Genetics suggest that FZD4 signaling plays a critical role in the development and maintenance of retina vessels and there remains a critical unmet need for retinopathy treatment.**
- **SZN-413 reduced avascular areas and pathologic neovascular tuft formation in a mouse model of OIR.**
- **SZN-413 reduced retinal vascular leakage in a rabbit model of VEGF-induced retinal vascular leakage.**

Conclusion

SZN-413 can simultaneously address the retinal nonperfusion and leakage characteristics of DR pathology addressing the unmet need and opening new possibilities for the treatment of DR.