

# Disrupting Angiofibrotic Retinal Signaling Through Nanoparticle-Based NADPH Oxidase Inhibition

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**INTRODUCTION:** Angiofibrotic retinal diseases, including neovascular age-related macular degeneration (nAMD), remain a leading cause of vision loss worldwide. Despite first-line intravitreal anti-VEGF therapy, up to 56% of patients develop subretinal fibrosis and irreversible vision loss within five years, warranting research into alternative therapies. NADPH oxidases are major generators of reactive oxygen species (ROS) and can modulate angiogenic and fibrotic signaling pathways, including endothelial-to-mesenchymal transition (EndMT). This study evaluated the efficacy of a non-selective NADPH oxidase inhibitor; VAS2870. Due to its hydrophobicity, acetalated dextran (AcDex) nanoparticles (NPs) were synthesized to improve the bioavailability for intravitreal delivery. **METHODS:** VAS2870 was loaded into AcDex NPs using a single emulsion method. Primary human retinal endothelial cells (HRECs) were treated with free VAS2870 (8 $\mu$ M), VAS2870-loaded AcDex NPs (equivalent concentration), and either pro-fibrotic TGF- $\beta$ 2 (10 ng/ml) or pro-angiogenic VEGF (10 ng/ml). Angiogenic responses were assessed by tube formation over 16 hours (n=6), with cell viability confirmed with calcein AM. Expression of EndMT markers ( $\alpha$ -SMA, vimentin) and the endothelial marker VE-cadherin was quantified by immunofluorescence following 72-hour treatment (n=3). Cell migration was assessed using a scratch wound assay over 24 hours (n=6). Mitochondrial ROS levels were measured using MitoSOX over 24 and 72 hours (n=3). **RESULTS:** VAS2870 was successfully encapsulated into AcDex NPs with an average size of ~200 nm. Free VAS2870 and VAS2870-loaded AcDex NPs significantly reduced tube formation and cell migration compared with untreated, VEGF- and TGF- $\beta$ 2-treated HRECs. TGF- $\beta$ 2 induced a mesenchymal phenotype characterized by upregulated  $\alpha$ -SMA and vimentin and downregulated VE-cadherin expression. VAS2870 attenuated pro-fibrotic marker expression and restored endothelial marker levels, consistent with inhibition of TGF- $\beta$ 2-mediated EndMT. Notably, VAS2870 was associated with an increase in mitochondrial ROS generation at 24 and 72 hours. **CONCLUSIONS:** Non-selective NADPH oxidase inhibition with free and NP-encapsulated VAS2870 demonstrates anti-angiogenic and anti-fibrotic activity in retinal endothelial cells, supporting further *in vivo* evaluation as a novel therapy for nAMD. The redox crosstalk between NADPH oxidases and mitochondrial ROS remains complex and requires further mechanistic investigation.