Mitochondrial-specific antioxidant XJB-5-131 attenuates endogenous TGF-β2 expression in human trabecular meshwork cells

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INTRODUCTION

Glaucoma is a leading cause of blindness, expected to affect nearly 80 million people worldwide by the year 2020. In the US, it is estimated that nearly 2 million individuals age 45 years and older currently have primary open angle glaucoma (POAG), the most prevalent form of the disease. Despite its overwhelming prevalence and socioeconomic impact, the treatment of patients with POAG remains restricted to non-specific interventions aimed at lowering intracocular pressure (IOP), a poorly understood hallmark of POAG.

In healthy eyes, IOP is maintained through balanced production and outflow of aqueous humor (AH). In humans, more than 50% of AH leaves the eye through the conventional outflow system, including the trabecular meshwork (TM). Under physiologic conditions, abnormally high IOP leads to mechanical stress and damage to tissues present in the AH outflow pathways.

TGF-β2, a member of the transforming growth factor-β (TGF-β) superfamily, is expressed and secreted by the TM cells, with elevated expression seen in TM cells from POAG eyes. The mechanisms underlying endogenous TGF-β2 expression and release remain unclear.

Recently, production of reactive oxygen species (ROS) has also been linked with the pathophysiology of POAG, the most prevalent form of the disease. Despite its overwhelming prevalence and socioeconomic impact, the treatment of patients with POAG remains restricted to non-specific interventions aimed at lowering intracocular pressure (IOP), a poorly understood hallmark of POAG.

Multiple studies have demonstrated that transforming growth factor (TGF)-β2, an anti-inflammatory cytokine that promotes ECM deposition and actin stress fiber organization in TM cells, is markedly elevated in the AH of patients with POAG. Despite this growing body of evidence supporting a causal role of TGF-β2 in the pathophysiology of POAG, the mechanisms underlying endogenous TGF-β2 expression and release remain unclear.

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METHODS

Porcine Anterior Segment Perfusion: Anterior segment perfusion experiments are performed using fresh porcine eyes obtained from a local abattoir. GLOBES are bisected aseptically at the equator, and the iris, lens, and vitreous gently removed. The prepared anterior segments are warmed DMEM supplemented with antibiotics and antimycotics. Anterior segments are perfused with either vehicle (400 nM HCl) or recombinant active human TGF-β2 (0.01% DMSO) or the proprietary mitochondria-targeted antioxidant XJB-5-131 (25 µM) and analyzed by two-way ANOVA with Bonferroni’s multiple comparison post-hoc analysis. Cell culture data are expressed as the means ± SD or SEM and analyzed using one-way ANOVA followed by Tukey’s multiple comparison post-hoc analysis. Statistical significance was defined as *p < 0.05.

RESULTS

A significant reduction in TGF-β2 expression and release was observed with the treatment of TGF-β2 with XJB-5-131 compared to vehicle treatment. The mean ± SD of TGF-β2 expression in vehicle-treated cells was 1.5 ± 0.2 pg/ml, while the mean ± SD of TGF-β2 expression in XJB-5-131-treated cells was 0.5 ± 0.1 pg/ml, achieving statistical significance (p < 0.05).

SUMMARY/CONCLUSION

XJB-5-131 is a bi-functional antioxidant featuring a radical scavenger 4-hydroxy-2,2,6,6-tetramethyl piperidine-1-oxyl nitrosoyl conjugated to an allene peptide that operates as a molecular stabilizer of the mitochondrial membrane (Wu et al., 2003).

ACKNOWLEDGMENTS

Supported, in part, by a Loyola University Chicago STAR award (M.N.C.), as well as grants from the Illinois Society for the Prevention of Blindness, the Department of Veterans Affairs and the Midwest Eye-Banks, and the Richard A. Perritt Charitable Foundation.

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Mitochondrial-targeted antioxidant XJB-5-131 may lower IOP by attenuating constitutive TGF-β2 expression and secretion within the conventional outflow pathway.