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Discovery of new agents to inhibit scarring and improve the success of glaucoma surgery

The glaucomas are a heterogeneous group of optic neuropathies sharing similar clinical features including cupping of the optic disc, thinning and loss of the retinal nerve fiber layer, and characteristic visual field defects[1]. A variety of risk factors have been identified for the development of glaucoma including elevated intraocular pressure (IOP), age, family history, central corneal thickness, and steroid responsiveness. IOP is the most significant causative risk factor for both the development and progression of glaucoma. Not all ocular hypertensive individuals develop glaucoma, but lowering IOP decreases the risk for developing glaucoma[2] and decreases glaucoma progression both early[3] and late[4] in the disease.

IOP is regulated by aqueous humor (AH) production and drainage from the eye. The trabecular meshwork (TM) is well known to be a critical tissue in aqueous humor drainage. The TM imparts a normal resistance to AH outflow that becomes abnormally increased in glaucoma. Trabeculectomy, or glaucoma filtration surgery, is a surgical operation that lowers the IOP inside the eye in patients with glaucoma. This is achieved by the formation of an artificial drainage route from the anterior chamber to the subconjunctival space. Surgically, a small hole is made in the eye wall (sclera), covered by a thin "trap-door". The aqueous humor drains through the trap-door to a small reservoir or bleb just under the eye surface, hidden by the eyelid. The success of the surgery significantly depends on postoperative healing. One of the major complications of trabeculectomy surgery is scar formation. Excessive scarring closes the surgically generated pathway for the aqueous humor, which then leads to a reoccurrence of high IOP.

Experimental animal models of glaucoma filtration surgery have previously been used to study IOP regulation and evaluate potential pharmacological modulators of wound healing. However, to date, the available drug therapies to prevent scar formation come with great side effects and limited success. The involvement of TGFβ signaling pathways in the regulation of the extracellular matrix (ECM) and scar formation has been extensively studied. Recent evidence has implicated toll-like receptor 4 (TLR4) and TGFβ2 signaling crosstalk in the regulation of ECM and fibrogenesis in other tissues such as liver, kidney, lung, and skin. Here we propose that the TGFβ2-TLR4 signaling crosstalk is also involved in the regulation of Tenon's capsule and scleral scar formation after glaucoma filtration surgery. ***Our hypothesis is inhibition of the TGFβ2-TLR4 signaling pathway will decrease scar formation after filtration surgery and maintain the reduced IOP.***

Specific Aims: We will address this hypothesis with 3 specific aims:

Specific Aim #1: Determine whether inhibition of TGFβ2-TLR4 signaling molecules regulates cell proliferation and migration in primary human and primary rabbit Tenon's capsule fibroblast cells, as well as in primary human and primary rabbit scleral fibroblast cells in culture. We will test four specific inhibitors. We will utilize a selective TLR4 inhibitor, TAK-242, and a selective MyD88 inhibitor, NBP2-29328, to inhibit TLR4 dependent signaling. We will also test a peptide mimetic of BMP-7, THR123. BMP-7 is an endogenous inhibitor of the TGFβ2 signaling pathway. Lastly, we will test anti-GREM1 antibodies. GREM1 is a known agonist of TGFβ2 signaling and its expression is increased in the anterior chamber of glaucoma eyes. As a positive control we will utilize Mitomycin-C (MMC), which is used clinically in glaucoma filtration surgery to inhibit scar formation. We will quantify cell viability using an MTT Assay, which is a colorimetric assay for assessing cell metabolic activity. Cell proliferation with be tested using a BrdU Test,

which incorporates BrdU into cellular DNA during cell proliferation and can be detected using an anti-BrdU antibody. Lastly, cell migration will be evaluated using a wound scratch assay.

Specific Aim #2: Determine whether inhibition of TGFβ2-TLR4 signaling molecules can prevent scar formation and maintain the reduced IOP in a rabbit glaucoma filtration model. Filtration surgery will be performed in one eye of each Dutch Belted rabbit (DB), with the contralateral eye serving as a naïve control (10 rabbits will be utilized per group). DB rabbits maintains higher IOP pre and post surgery compared to other rabbit strains, such as the New Zealand White rabbits, which will help provide more defined outcome measures[5]. Following surgery, a selective TLR4 inhibitor, TAK-242, a selective MyD88 inhibitor, NBP2-29328, a peptide mimetic of BMP-7, THR123, or anti-GREM1 antibodies, will be applied to the surgical site by topical ocular administration. Silver nanoparticles (AgNPs)[6] and MMC will be used as positive controls and/or to extend the durations of the inhibitors actions. Intraocular pressure will be monitored using a TonoVet tonometer before, immediately after surgery, and two-times a week for 4 weeks post-surgery. 4-weeks post-surgery the animals will be sacrificed and histopathology and immunochemistry techniques will be performed on the eyes to determine the amount of scarring and fibrosis using markers for fibrotic proteins such as Masson trichrome staining, anti-fibronectin, anti-collagen, and anti-smooth muscle actin antibodies.

Specific Aim #3: Determine whether TGFβ signaling molecules are differentially expressed after filtration surgery in rabbits. Filtration surgery will be performed in one eye of each DB rabbit, with the contralateral eye serving as a naïve control (10 animals/experiment). Aqueous humor samples will be removed from each eye 1-week before filtration surgery, 1-week post filtration surgery, and 4-weeks post-filtration surgery. TGFβ1, TGFβ2, and TGFβ3 expression levels will be assessed in the aqueous humor samples using a TGF-β 3-plex ImmunoAssay (Bio-Rad).

References

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Budget

Rabbit model of trabeculectomy: $26,000
    Dutch Belted Rabbit = $120/animal
    Animal per diem = $1.98/day/animal
Laboratory reagents/surgery tools: 10,000
Research Associate 40% effort: $24,000

Total Budget $60,000