

THE EFFECTS OF BEVACIZUMAB ON HUMAN
TENON'S FIBROBLASTS: AN *IN VITRO* STUDY

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ABSTRACT

Purpose: To understand the effect and mechanism of bevacizumab in inhibiting wound healing on primary human Tenon's capsule fibroblasts (HTF) in vitro.

Methods: HTF was cultured in RPMI media and bevacizumab was added to study the effect of bevacizumab on HTF in vitro at a concentration of 0.25,1.25,6.25 and 12.5 mg/mL. The HTF morphology was identified using vimentin antibody and DAPI staining under immunofluorescent images. MTT assay was done to assess fibroblast cells viability and cell death. The results were compared with mitomycin C. The levels of caspase 3 and TGF β 2 protein expression were measured by ELISA.

Results: By MTT assay, bevacizumab induced apoptosis in HTFs in a concentration- and time-dependent manner. Both concentrations at 6.25 mg/mL and 12.5 mg/mL showed a significant fibroblast cells death, however the result was statistically more significant with 12.5 mg/mL ($p < 0.01$) than 6.25 mg/mL ($p < 0.05$). The protein level of caspase-3 was significantly upregulated to 2.5 folds with $p < 0.01$ (control: 7.0mg/mL, treated: 17.6 mg/mL) but TGF- β 2 expression also was significantly upregulated with $p < 0.01$ (control: 85.5mg/mL, treated: 341.7 mg/mL) .

Conclusion: Bevacizumab at concentration 12.5 mg/mL induces statistically significant human Tenon's fibroblasts cell death after an incubation period of 48 hours through caspase 3 pathway.

CHAPTER 1

INTRODUCTION

GLAUCOMA

Glaucoma is defined by the World Health Organization (WHO) as an ocular disorder characterized by structural changes and defect in the retinal ganglion cells (RCG) bodies and their axons which eventually over a period of many years will lead to permanent loss of vision.(WHO, 2013) The characteristic appearance of the optic nerve head such as thinning of the neuroretinal rim , optic-nerve cupping, disc hemorrhage and retinal nerve fiber layer death differs glaucoma from other optic neuropathies.(H. Kwon Young 2009) Currently, as the population grows older, the prevalence of glaucoma rises and it is estimated that by 2020, 79.6% of the world population will be suffering from glaucoma making it the leading cause of irreversible blindness globally. (Table 1) (Quigley H A 2006) Patients with glaucoma will be having a peripheral vision loss first causing them to have a tunnel vision and advances to involve the central vision. It also known as the “silent thief” of sight and a study showed that only 50% of people with glaucoma were diagnosed with the disease.(Anne, 1999) Usually, they will only seek for medical attention when they are already at terminal stage where 40% or more of the retinal ganglion cells are lost (Quigley HA, 1989) because in most cases the patients present with no symptoms making early detection of it is difficult.