UNIVERSITI TEKNOLOGI MARA

MECHANISMS OF RANIBIZUMAB AS AN ANTI-SCARRING AGENT IN TRABECULECTOMY

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PhD

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This topic has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I hereby acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Trabeculectomy is the gold standard procedure performed in glaucoma when topical medication and laser intervention fail to maintain the ideal intraocular pressure (IOP) of patient's eye. However, excessive accumulation of extracellular matrix components (ECM) mediated by Tenon's fibroblast (HTF) leads to significant cases of surgical failure. Anti-vascular endothelial growth factor (VEGF) has become the focus in current scar modulation strategy. Improved bleb morphology following trabeculectomy augmented with ranibizumab has been reported. However, mechanism of actions of ranibizumab on HTF is not well understood. Therefore, this *in vitro* study was conducted to elucidate mechanism of actions of ranibizumab on HTF. HTF used in this study were propagated from Tenon's capsule obtained from patients undergoing trabeculectomy. Firstly, isolated and characterized HTF were treated with different concentrations of ranibizumab in serum and serum-free media for 24 and 48 hours and then HTF viability was measured using MTT assay. Then, HTF were extracted to measure the expression of collagen Type 1 (COL1A1), fibronectin (FN), transforming growth factor-β1 and -β2 (TGF-β1 &TGF-β2) using qRT-PCR and ELISA. The experiment was followed with metabolomics profiling which was performed to identify the most significant metabolite regulated by ranibizumab. Finally, the expression of regulatory genes and proteins involved in the cell cycle regulation and angiogenesis including p21, p53, CDK2, CDK4, *PTEN*, *AKT1* and *THBS1* were measured by RT^2 Profiler PCR Array and Western Blot. Findings from the MTT assay showed that ranibizumab at the concentration of 0.5 mg/ml induce significant reduction in HTFs viability. The optimum degree of reduction was observed in serum-free media incubated for 48 hours. Furthermore, results suggested that ranibizumab mediates the down-regulation of COL1A1 and TGF-β1at gene level, but not at the protein level. No relevant changes were observed in FN and TGF-β2 mRNA level, but the proteins level was up-regulated. In metabolomics study, ranibizumab was shown to induce significant reduction in spermidine level. Therefore in subsequent experiment, ranibizumab effects were compared to DFMO, a potent irreversible inhibitor in spermidine synthesis. Findings show that ranibizumab exerts similar mechanism to DFMO in regulating spermidine expression by HTF, where it reduces *PTEN*, *AKT1* and *THBS1* expression. Moreover, ranibizumab administration increase p53 and p21 expression and reduces CDK2 and CDK4 expression. These observations suggest that ranibizumab might exert its anti-scarring property by enhancing the activities of p53 and p21, thus lead to reduction in CDK2 and CDK4. This shows that cell cycle of ranibizumab-treated HTF could be arrested, particularly at G1 phase.

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