UNIVERSITI TEKNOLOGI MARA

TIME AND DOSE DEPENDENT EFFECTS OF RENIN ANGIOTENSIN SYSTEM INHIBITORS ON EXTRACELLULAR MATRIX DEPOSITION AND MODULATING ENZYMES IN DEXAMETHASONE TREATED HUMAN TRABECULAR MESHWORK CELLS

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MSc

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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 thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

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ABSTRACT

Trabecular meshwork remodeling leads to aqueous humor outflow resistance and increases in intraocular pressure, one of the major risk factors for glaucoma. Trabecular meshwork remodeling is associated with excessive deposition of extracellular matrix components such as collagen, fibronectin, and α-smooth muscle actin, and reduction in the production of matrix metalloproteinases responsible for the extracellular matrix degradation. Inhibitors of the renin angiotensin system were shown to reduce intraocular pressure in animals and clinical studies. They are also well known to attenuate tissue remodelling in the cardiovascular system, however, their effects on human trabecular meshwork have not been investigated yet. The dose and time-dependent effects of RAS inhibitors on extracellular matrix in dexamethasone treated human trabecular meshwork cells, as well as the mechanisms involved in anti remodeling effects of the renin angiotensin system inhibitors were investigated in this study for the first time. The long-time effects of dexamethasone on human trabecular meshwork cells were investigated as well. Human trabecular meshwork cells were cultured in Dulbecco's Modified Eagle's medium, dexamethasone 10^{-7} M, or dexamethasone 10^{-7} M with enalaprilat dihydrate or losartan potassium in concentrations of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M. All groups were incubated for 7 and 14 days. The same grouping and the time points were used for the study of the effects of the renin angiotensin system inhibitors on human trabecular meshwork cells initially pretreated with dexamethasone for 14 days. MTS assay was done to evaluate cell viability. Immunocytochemistry, western blot, and ELISA were performed to measure extracellular matrix deposition, modulating enzymes, TGF-β2, uPA, and tPA levels. Dexamethasone significantly increased the production of fibronectin and α-smooth muscle actin compared to that in media only treated cells at both time points. At the same time, dexamethasone decreased the production of both MMP-2 and MMP-9. TIMP-1 and -2 levels were increased at both time points but reached the significantly higher levels only on day 14. The renin angiotensin system inhibitors significantly reduced production of fibronectin and α-smooth muscle actin in both dexamethasone co- and pretreated HTM cells. Both tested renin angiotensin system inhibitors also increased levels of MMPs. Antiremodeling effect of the renin angiotensin system inhibitors was seen in a dose-independent manner, and all tested parameters reached significantly different levels by week 2 of the treatment. The mechanism of attenuation dexamethasone-induced changes in the human trabecular meshwork cells was associated with significantly enhanced secretion of uPA and suppression of TIMPs secretion. The findings suggest that both groups angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are attractive as new options for the treatment and prevention of steroid-induced glaucoma as well as other forms of open angle glaucoma.

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