UNIVERSITI TEKNOLOGI MARA

THE EFFECTS OF *TRANS*-RESVERATROL ON TRANSFORMING GROWTH FACTOR-β2 SIGNALLING PATHWAYS VIA JNK AND p38 ON FIBRONECTIN DEPOSITION IN STEROID-TREATED HUMAN TRABECULAR MESHWORK CELLS

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MSc

April 2024

ABSTRACT

Glaucoma is the leading cause of irreversible blindness. Currently available treatments mainly target the reduction of intraocular pressure (IOP). Increased extracellular matrix (ECM) deposition especially fibronectin (FN) in trabecular meshwork (TM) contributes to aqueous humour (AH) outflow resistance, causing elevated IOP. Transforming growth factor-beta (TGF-β) is a cytokine implicated in the pathophysiology of glaucoma and its TGF-β2 isoform has a more significant association. It enhances ECM deposition in the TM tissue via connective tissue growth factor (CTGF) and the signalling may involve canonical, the SMAD-dependent and/or non-canonical pathways, via mitogen-activated protein kinase (MAPK) such as JNK and p38. Steroid-induced glaucoma, a common type of secondary glaucoma, is also associated with elevated IOP and TM changes similar to those observed in the most common glaucoma subtype, primary open angle glaucoma (POAG). *Trans*-resveratrol (TR) is a polyphenolic compound that has previously been shown to lower IOP in steroid-induced ocular hypertensive rat model. TR has been shown to minimally affect the canonical TGF-β2 signalling pathway in primary human trabecular meshwork cells (HTMCs). Therefore, this study aims to explore the involvement of non-canonical TGF-β2 signalling via JNK and p38 MAPKs leading to reduction of FN deposition by TR in dexamethasone-treated primary HTMCs. Primary HTMCs were incubated with dexamethasone (100 nM) with and without TR (12.5 μM). The gene and protein expressions of TGF-β2, JNK1, JNK2, p38, CTGF and FN were determined using RT-qPCR and ELISA after 3- and 7-days of treatment, respectively. The active TGF-β2, phosphorylated JNK1 and phosphorylated p38 were also measured using ELISA after 7 days. It was observed that TR downregulates dexamethasone-induced increase in the expression of TGF-β2 gene, and both total and active TGF-β2 proteins. Treatment with dexamethasone-only increased the phosphorylated JNK1 and p38, however it had insignificant effect on the gene and protein expressions of JNK1, JNK2 and p38. TR alone and TR co-treatment with dexamethasone had insignificant effect on JNK1 gene and protein expression and JNK2 gene expression when compared to both dexamethasone-only treated group and control groups. TR however when given alone and with dexamethasone reduced the JNK2 protein expression significantly when compared to dexamethasone-only group and control groups. TR co-treatment with dexamethasone increased JNK1 phosphorylation when compared to the control group and reduced the p38 expression and phosphorylation in comparison to dexamethasone-only group. TR co-treatment with dexamethasone also suppressed both CTGF and FN expression induced by dexamethasone treatment. This current study demonstrated that TR suppressed TGF-β2 pathway leading to reduction in CTGF and FN levels with involvement of p38 MAPK but not JNK. Further investigations are warranted to fully understand the mechanism of action of TR underlying reduction of ECM deposition and its potential use as an antiglaucoma agent.

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious, the Most Merciful. My utmost gratefulness to Allah the Almighty, with His love and blessings, this MSc journey is actually coming to an end. For the past three years, I had been blessed with great health and unwavering supports.

Firstly, I would like to express my sincere gratitude to my supervisor, Dr. Norhafiza Razali for giving me the opportunity to conduct this research for my MSc. Although I had little experience when I started out, her enthusiasm ignited my hopes and determination. Even though I encountered numerous hurdles throughout my lab work, I was never left out, and it was thanks to her tireless guidance and morale that I managed to stand on my feet again. She is the ideal main supervisor I could have ever asked for. I am equally grateful to my co-supervisors, Prof. Dr. Renu Agarwal and Dr. Noor Fahitah Abu Hanipah for their insightful ideas, intellectual abilities and inspiring quotes. Without my supervisors' wisdom and expertise, I would not be able to resolve any difficulties that I have endured. I aspire to be a remarkable academician like them.

My gratitude is extended to the members of Department of Pharmacology, Prof. Dr. Igor, Dr. Zulfiqah, Normie, Afiqq, and Amy for their compassionate and constant guidance on scientific studies. I am also extremely thankful to Hafidz, Wafriy, Fatihah, Auni, Syaza, Farrah, Sarah, Azirah, Amirah, Melonney, Johanes, Suhana, Muhaimin and Sharifah, my fellow postgraduate seniors and colleagues who are also pursuing research in diverse disciplines. My research work was immensely effective and productive thanks to their mentorship, knowledge sharing, exchange of tips, precautionary advice and wise counsel.

My heartfelt gratitude goes to Hazwan, who was there for me through my ups and downs and had faith in everything I do. The person with whom I feel at ease going over any of my personal thoughts, feelings, fears and excitements. Special thanks to my other undergraduate juniors, Aqif and Hamzi, who treated me well and lightened my days. They were continually intrigued and concerned about my research progress and my well-being, despite having a lot on their plate for their studies and exams. Not to mention my closest peers, Arshad, Atiqah, Hafizan, Faris, Murali, Aiman and Syarizzat, who are now in their finest careers. Thank you for being supportive and encouraging in my quest for furthering my educational pursuits.

My profound thanks to the UiTM staffs from the Faculty of Medicine and Institute of Medical Molecular Biotechnology (IMMB) for providing their well-equipped facilities, guidance as well as organized events and workshops. I gratefully acknowledge the funding source that made my MSc work possible. The work was funded by Ministry of Higher Education Malaysia under grant number 600-IRMI/FRGS 5/3 (413/2019).

Last but not least, my deepest appreciation to my mother, Zuridah for supporting my academic route and providing financial assistance. This thesis serves as a tribute to her and my late father, Hassan for their unyielding dedication in raising and educating me. I hope this accomplishment has inspired my siblings, relatives and friends to keep acquiring and exploring new knowledge.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Glaucoma is a common eye disorder that can cause progressive optic nerve damage leading to a permanent visual loss. It is currently the leading cause for irreversible blindness worldwide with a prevalence of 2.4% for primary open angle glaucoma (POAG) affecting around 68.56 million people globally with 53% of those affected are in Asia (Zhang et al., 2021). Glaucoma can be classified into primary and secondary types. Primary glaucoma can be further classified into open-angle and closed-angle glaucoma. Secondary glaucoma can be caused by trauma, inflammation, tumour and medications such as corticosteroids (Weinreb et al., 2014).

Increased intraocular pressure (IOP) is an important risk factor for glaucoma development and progression. IOP is the tissue pressure of the intraocular content and is kept in the normal range by a balance between the rate of secretion of aqueous humour (AH) from the ciliary body and rate of its drainage through a heterogenous tissue, the trabecular meshwork (TM), into the Schlemm's canal. Increased extracellular matrix (ECM) deposition such as fibronectin (FN) in TM tissue causes increased aqueous outflow resistance leading to IOP elevation in glaucoma (Tektas et al., 2010; Vranka et al., 2015; Kasetti et al., 2018). Ocular hypertension or IOP elevation progressing to glaucoma can also develop as an adverse effect from corticosteroid therapy, either topical or systemic, administered for a sufficient duration (Kersey & Broadway, 2006). Currently available treatment modalities for glaucoma target to reduce the IOP which helps to reduce the development and progression of the disease (Heijl et al., 2002; Razali et al., 2021).

Transforming growth factor-beta (TGF-β) belongs to TGF superfamily and is a cytokine with multiple functions and has been implicated in glaucoma pathophysiology. A meta-analysis by Agarwal et al., (2015) showed significantly higher level of TGF-β2 in AH of POAG patients when compared to controls. TGF-β2 contributes to the elevation of IOP, in part, as a result of enhanced ECM deposition contributing to resistance to AH outflow in the TM tissue. Exposure to TGF-β2 has been shown to significantly enhance the synthesis and deposition of multiple ECM and ECM-related