UNIVERSITI TEKNOLOGI MARA

CELLULAR AND SUBCELLULAR MECHANISMS OF ACTION OF RESVERATROL IN REGULATION OF AQUEOUS HUMOUR DYNAMICS

NORHAFIZA BINTI RAZALI

Thesis submitted in fulfilment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Medicine

March 2016

AUTHOR'S DECLARATION

I declare that the work in this dissertation 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.

Name of Student	:	Norhafiza Binti Razali
Student I.D. No.	:	2011271998
Programme	:	Doctor of Philosopy (Pharmacology) – MD990
Faculty	:	Faculty of Medicine
Thesis Title	:	Cellular and Subcellular Mechanisms of Action of Resveratrol in Regulation of Aqueous Humour Dynamics
Signature of Student	:	…….
Date	:	

ABSTRACT

Glaucoma, a common cause of optic neuropathy, is associated with elevated intraocular pressure (IOP) and is the leading cause of irreversible visual disability. Steroid-induced glaucoma, a common type of secondary glaucoma, is also associated with elevated IOP (SIOH). SIOH and glaucoma are currently treated with antiglaucoma agents, which often have suboptimal efficacy and are associated with adverse effects. The objective of this study was to determine if topical application of *trans*-resveratrol reduces IOP in rats with SIOH and to investigate its mechanisms of action. This study was divided into in vivo and in vitro studies. In the in vivo study, we evaluated the oculohypotensive effects of topical *trans*-resveratrol in normal and SIOH rats and investigated the role of adenosine receptors (ARs) and transforming growth factor-β (TGF-β) signalling in the IOP lowering effect of *trans*-resveratrol. Involvement of AR was studied by observing the alterations of IOP in response to *trans*-resveratrol after pre-treating SIOH animals with AR subtype-specific antagonists. The study also investigated phospholipase C (PLC) activation, extracellular regulated kinase 1/2 (ERK1/2) phosphorylation and increased matrix metalloproteinases (MMPs) secretion in the aqueous humour (AH) as mechanism of resveratrol-induced oculohypotension in SIOH rats. In vitro studies evaluated the effect of *trans*-resveratrol on cellular signalling pathways of ARs and TGF-β in primary human trabecular meshwork cells (HTMCs). Single drop of all concentrations of *trans*-resveratrol produced significant oculohypotension in normotensive rats and 0.2% concentration produced maximum IOP reduction. Twice daily topical application of *trans*-resveratrol 0.2% for 21-day in SIOH rats resulted in significant and sustained IOP reduction. This was associated with significantly higher AH MMP-2 level; significantly reduced trabecular meshwork (TM) thickness and increased number of TM cells. Treatment with *trans*-resveratrol also significantly increased the ganglion cell survival and reduced retinal oxidative stress. Pretreatment with adenosine A_1 receptor antagonist inhibited the oculohypotensive effect of resveratrol. The use of A_1 AR, PLC and ERK 1/2 inhibitors also reduced resveratrol-induced MMP-2 secretion. These results were further supported by in vitro study that demonstrated that ERK1/2, PLC and MMP-2 secretion by HTMC is stimulated after resveratrol treatment and these effects are associated with upregulation of A_1AR gene expression. Topical *trans*-resveratrol also produced significantly raised plasminogen activator levels and combined TGF-β2+resveratrol treatment caused significant upregulation of inhibitory SMAD7 when compared to TGF-β2-only treated group. Hence, it could be concluded that *trans*-resveratrol-induced oculohypotension in SIOH rats involves its agonistic activity at the A_1AR leading to PLC activation, ERK 1/2 phosphorylation and increased MMP-2 secretion. Increased MMP-2 secretion seems to cause changes in TM favourable for AH outflow leading to reduced IOP. *Trans*-resveratrol-induced oculohypotension could also be attributed to increased level of plasminogen activators, which seems to result from increased expression of inhibitory SMAD7, a TGF-β2 signalling molecule. Although current study, for the first time, has clearly demonstrated the significant effects of topical *trans*-resveratrol on IOP in rats with SIOH and some of the underlying mechanisms; further investigations are needed to fully understand the mechanisms of action of *trans*-resveratrol and to explore its potential as a future 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 PhD journey is actually coming to an end. I had been blessed with good health, good family and good support for the past 4 years.

There is not enough thank you that I can ever express to my supervisor Assoc. Prof. Dr Renu Agarwal. The best supervisor, mentor, mother, colleague, friend, I could have ever wished for. You have been there together with me through thick and thin from registering as a Masters' student, converting to PhD and finally completing this thesis. You pushed me so I can achieve extraordinary things. Thank you Dr Renu. I cannot repay you for everything that you have done for me. I am indebted for life for this knowledge and guidance and I hope I will be a supervisor and a mentor as good as what you have been to me.

Sincere thanks goes to my other mentors and co-supervisors. I thank both Prof. Dr Nafeeza Mohd Ismail and Assoc. Prof. Dr Gabriele Anisah Froemming for your guidance in this research and writing throughout the challenging journey. My heartfelt gratitude to Dr Minaketan Tripathy from Faculty of Pharmacy for his help in dissolving my chemical for the project when I almost lost hope. Thank you so much to Dr Puneet Agarwal for teaching me all the techniques in ocular research, being there in the animal lab to teach me all those methods as well as your input in all of my paper publications and abstract proceedings.

I gratefully acknowledge the funding sources that made my PhD work possible. The work was funded by Ministry of Higher Education Malaysia under grant number 600-RMI/ST/FRGS.5/3/Fst (50/2011), 600-RMI/RAGS 5/3 (104/2013), 600-RMI/RAGS 5/3 (39/2014). Furthermore, my study was made possible by the scholarship provided by Ministry of Higher Education Malaysia and Universiti Teknologi MARA (UiTM) as part of the Skim Latihan Academik Bumiputera (SLAB).

Thank you to all my friends and the staffs from IMMB and LACU for helping me in many ways and to both my parents for supporting me in whatever career path that I have chosen and always encouraging me to never give up. I dedicate this thesis to both of you; Razali Mohd Nor and Zawiah Muhammad, for your endless love and support.

Last but not least to my heroes Halami Aman Mohd Mokhtar and Muhammad Umar Halami Aman, I love you both to the moon and back. Thank you to my dear husband for letting me embark on what seemed to be an endless journey, caring for our little one when I had to spend time in the lab or writing. For my dear son, Ummi hope that I have inspired you to always be eager for knowledge and to always learn.

Alhamdulillah.

TABLE OF CONTENTS