UNIVERSITI TEKNOLOGI MARA

PROTECTIVE EFFECT OF *TRANS*-RESVERATROL ON DEXAMETHASONE-INDUCED CHANGES IN MATRIX METALLOPROTEINASES EXPRESSION BY HUMAN TRABECULAR MESHWORK CELLS: POTENTIAL MECHANISMS

NORMIE AIDA BINTI MOHD NASIR

MSc

July 2019

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.

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	:	Normie Aida binti Mohd Nasir
Student I.D. No.	:	2015225328
Programme	:	Master of Medical Science (Pharmacology) – MD758
Faculty	:	Medicine
Thesis Title	:	Protective Effect of Trans-resveratrol on Dexamethasone-induced Changes in Matrix Metalloproteinases Expression by Human Trabecular Meshwork Cells: Potential Mechanisms
Signature of Student	:	……..
Date	:	July 2019

ABSTRACT

Matrix metalloproteinases (MMPs) can regulate extracellular matrix (ECM) turnover in the trabecular meshwork (TM) of eye. Reduced MMP secretion increases ECM deposition in TM, which is associated with increased intraocular pressure (IOP). Increased IOP is the major risk factor for glaucoma, the leading cause of irreversible blindness, worldwide. Previously *trans*-resveratrol was shown to reduce IOP in normotensive and oculohypertensive rats. The dose- and time-dependent effects of *trans*-resveratrol on human TM cells (HTMCs) viability, MMP-2 and -9 secretion and the mechanisms involved remain unknown and hence these effects were investigated in the present study. HTMCs were cultured and divided into 11 groups that received treatment with DMSO (0.1%), dexamethasone (100 nM), and *trans*-resveratrol (3.125, 6.25, 12.5, 25 and 50 μM) either in the presence or absence of dexamethasone for 2, 5 and 7 days. To study the role of A_1 adenosine receptors (A_1AR) and nuclear factor kappa B (NFκB) in *trans*-resveratrol mediated effects on MMP secretions, additional groups of HTMCs were pre-treated with dipropylcyclopentylxanthine (DPCPX), a specific A_1AR antagonist and curcumin, an inhibitor of NFκB. Cell viability was determined by using MTS assay while MMP-2 and -9 expressions were investigated using western blot. A_1AR expression was determined using western blot and ELISA while NFκB activation was determined using immunocytochemistry and ELISA. This study demonstrated that incubation of HTMCs with *trans*-resveratrol up to a concentration of 25 μM does not affect the viability but at 50 μM, it significantly reduces viability of HTMCs both in the presence and absence of dexamethasone. This effect of *trans*-resveratrol on the viability of HTMCs was dose-dependent but not time-dependent. Dexamethasone reduced MMP-2 and -9 expressions after 5 and 7 days treatment, compared to untreated group ($p<0.01$). Incubation with 12.5 μM *trans*-resveratrol for 5 days in the presence of dexamethasone increased MMP-2 and -9 level compared to dexamethasone-treated group ($p<0.05$). Significant reduction of A_1AR expression was seen in dexamethasone-treated group compared to untreated group ($p<0.01$). *Trans*-resveratrol significantly increased A_1AR expression compared to dexamethasone treated and DPCPX-treated groups ($p<0.001$). Dexamethasone significantly reduced NFκB activation compared to the untreated group ($p<0.001$). Increased nuclear localization of phospho p65 NFκB was seen in *trans*-resveratrol treated group in the presence and absence of dexamethasone. In conclusion, *trans*-resveratrol counteracts the effects of dexamethasone on MMP-2 and -9 levels of HTMCs, which could be mediated by A_1AR and NFκB.

ACKNOWLEDGEMENT

First of all, I would like to express my gratitude to Allah the Almighty for the opportunity to work in this field and complete the study successfully.

I am taking this opportunity to sincerely thank all of my supervisors, Professor Dr Renu Agarwal, Associate Professor Dr Anna Krasilnikova and Associate Professor Dr Siti Hamimah Sheikh Abdul Kadir for all the advices, constant encouragement and careful monitoring for the studies. I was also very fortunate that I had a kind association with the Pharmacology team members (Ainsya, Natasha, Lidawani, Azliana, Aizuddin and Adrian) that provided huge amount of priceless time and help to me. Special thanks to Associate Professor Dr Igor Iezhitsa, Dr Norhafiza Razali, Dr Noor Fahitah Abu Hanipah, Dr Nurul Alimah Abdul Nasir and Dr Siti Norsyafika Kamarudin for all the tips and experiences that we shared throughout this study.

To all friends, colleagues, Science Officers and staff of Institute of Molecular and Biotechnology (IMMB) and Clinical Training Centre (CTC), thank you for all the generous attitude and friendly support. They are truly wonderful people and I will never forget the days I spent in UiTM.

I would like to thank my mother and family members for the endless love, emotional and financial support throughout the journey to complete my studies. Last but not least, there are not enough words to describe just how important he was to me, therefore, this thesis is dedicated to my late father, En. Mohd Nasir Yusoff.

TABLE OF CONTENTS

		Page
CONFIRMATION BY PANEL OF EXAMINERS		**ii**
AUTHOR'S DECLARATION		**iii**
ABSTRACT		**iv**
ACKNOWLEDGEMENT		**v**
TABLE OF CONTENTS		**vi**
LIST OF TABLES		**xi**
LIST OF FIGURES		**xii**
LIST OF ABBREVIATIONS		**xv**
CHAPTER ONE: INTRODUCTION		**1**
1.1	Research Background	1
1.2	Problem Statement	3
1.3	Objectives	4
	1.3.1 General Objective	4
	1.3.2 Specific Objectives	4
1.4	Hypotheses	4
1.5	Significance of Study	5
1.6	Scope and Limitations of Study	5
CHAPTER TWO: LITERATURE REVIEW		**6**
2.1	Glaucoma	6
	2.1.1 Definition and Classification	6
	2.1.2 Prevalence	7
	2.1.3 Risk Factors	7
2.2	Ocular Structure and Intraocular Pressure (IOP)	8
	2.2.1 Gross Structure of Eye	8
	2.2.2 Aqueous Humour (AH)	9
	2.2.2.1 Formation and circulation	10
	2.2.2.2 Outflow	11