

UNIVERSITI TEKNOLOGI MARA

**MODULATION OF INFLAMMATION
AND ENDOTHELIAL ACTIVATION
WITH SPACEFLIGHT TRAVEL:
TOCOTRIENOLS AS
ATHEROPROTECTIVE AGENTS**

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

Faculty of Medicine

December 2013

ABSTRACT

The effects of immediate spaceflight travel on inflammation and endothelial activation in human endothelial cells (ECs) is not yet established. In addition, the expression of these biomarkers in revived live ECs recovered from a spaceflight travel has not been reported so far. Endothelial activation is preventable. One of the major preventive strategies is the usage of antioxidants. Tocotrienols (TCTs) is a more potent antioxidant than tocopherol (TOC). However, the role of Tocotrienol enriched mixed fraction (TEMF) and pure TCT isomers as a potential potent anti-atherosclerotic agent in human ECs compared to pure α -TOC is not well established. The anti-atherosclerotic mechanism of TCTs is also unclear. The objectives of this study were to investigate (i) the effects of spaceflight travel on the protein and gene expression of inflammation and endothelial activation, nuclear factor kappa B (NF κ B) and endothelial nitric oxide synthase (eNOS) in human ECs compared to ground controls, (ii) the protein and gene expression of inflammation and endothelial activation, NF κ B, signal transducer and activator of transcription-3 (STAT-3) and eNOS in revived live human ECs compared to matched controls (iii) the effects of TEMF, pure TCT isomers, and α -TOC on inflammation, endothelial activation, monocytes binding activity, NF κ B and eNOS, and (iv) the most potent pure TCT isomers on the inhibition of the inflammation, endothelial activation, monocytes binding activity, NF κ B and eNOS biomarkers in lipopolysaccharides (LPS) stimulated human ECs. The culture medium and ECs from post-spaceflight, revived and corresponding controls were collected and measured for protein and gene expression of cytokines (IL-6 and TNF- α), adhesion molecules (ICAM-1, VCAM-1 and e-selectin), NF κ B and/or STAT-3 and eNOS. Human umbilical vein endothelial cells (HUVECs) were incubated with various concentrations of TEMF, pure TCT isomers and α -TOC (0.3-10 μ M) together with, lipopolysaccharides (LPS) for 16 hours. Culture medium and cells were collected and measured for the protein and gene expression of cytokines, adhesion molecules, NF κ B and eNOS. The immediate post-spaceflight cells showed enhanced expression of cytokine (IL-6), adhesion molecules (ICAM-1 and VCAM-1) and NF κ B compared to ground controls. Following post spaceflight, the revived cells were shown to have increased expression of IL-6, ICAM-1 and STAT-3. TEMF and pure TCT isomers reduce IL-6, ICAM-1, VCAM-1, e-selectin, monocytes binding activity, NF κ B and induce eNOS expression. Area under the analysis revealed that pure TCT, particularly γ - and δ -isomers have better reduction of inflammation and endothelial activation and greater eNOS increment than TEMF. Delta (δ)-TCT is the most potent TCT isomers in terms of as an atheroprotective agent. Spaceflight travel leads to enhanced inflammation and endothelial activation and these remain elevated even after 3 months post spaceflight travel. This study provided a better understanding on the modulation of inflammation and endothelial activation associated with space travel and may direct future studies in the prevention of atherosclerosis in space travel. TEMF and pure TCT isomers exhibit anti-atherosclerotic properties with great potential as atheroprotective agents. The possible pathway for its anti-atherosclerotic activity is through the NF κ B deactivation. α -TOC has inhibitory effects on the anti-atherosclerotic properties of TCTs in TEMF.

ACKNOWLEDGEMENT

In the Name of Allah, The Most Gracious and The Most Merciful. Alhamdulillah. I would like to extend my sincere gratitude and deepest appreciation to a number of people, without whom this thesis would not be possible. Particularly, to my supervisor, Prof. Dr. Hapizah Mohd. Nawawi, for her invaluable guidance and encouragement throughout this research and preparation of thesis. My sincere thanks also dedicated to the members of my supervisory committee, Assoc. Prof. Dr. Gabriele Anisah Ruth Froemming and Prof. Dr. Abd. Manaf Ali for their professional assistance, useful suggestions and extensive discussion throughout this research process.

My deepest gratitude to Young Lecturer Scholarship Scheme under Universiti Teknologi MARA (UiTM), Shah Alam, Malaysia, for the scholarship. My special thanks to the Centre of Pathology Diagnostic and Research Laboratories (CPDRL) and Institute of Medical Molecular Biotechnology (IMMB), Faculty of Medicine, UiTM Sungai Buloh Campus for the excellent research facilities. My deepest appreciation to Mrs. Norita Salim from the IMMB for being a good companion and the best team-mate ever throughout our journey in the Space Mission experiment. This appreciation also dedicated to all my laboratory friends especially to Miss Azlina A. Razak, Mrs. Rafezah Razali and Miss Rahayu Izanwati for always sharing their thoughts, guidance and motivations throughout my journey in finishing this research project. My special thanks also to Dr. Mohdzir Mohd. Yasin for the guidance and good suggestions in improving my thesis writing.

My biggest appreciation to my beloved husband, Hasry Husairy b. Abd Hedi and my children, Hafiy Hazim and Hediya Hanna. Thank you for the prayers, encouragement and being the biggest supporters. My special appreciation to my beloved parents, Abd. Muid b. Abd. Rashid and Hajrah bt. Abu Shah and my beloved brothers, Shahrul Hady b. Abd. Muid and Shahir Amir b. Abd. Muid for their prayers, financial support and motivation. My special thank also dedicated to my parents in law and families. Without these important peoples in my life, I would not be able to finish this research and thesis successfully. All of you are the greatest gift of Allah to me.

I would like to take this opportunity to thank the Malaysian Ministry of Science, Technology and Innovation (MOSTI) under the Biotechnology Fund for the research grants, Faculty of Medicine, UiTM, Malaysian Space Agency (ANGKASA), Institute of Biomedical Problem (IBMP), Moscow and Institute of Postgraduate Studies (IPSIS), UiTM, Shah Alam. With this research, I have made a lot of good friends and gain a lot of precious knowledge. During the Malaysian Scientific Space Mission Programme, I have managed to obtain invaluable experience dealing with great and professional individuals International and Nationally. I hope to continue in giving my full commitment in research and contribute to significant findings especially in the prevention of atherosclerosis.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND AND PROBLEM STATEMENTS

Atherosclerosis is a slowly progressing disease of the medium and large sized arteries and characterized by formation of fatty and fibrous lesions in the vessel wall (Winther et al., 2005). As a result, it will lead to serious atherosclerosis-related clinical complications such as stroke, peripheral vascular diseases (PVD) and cardiovascular diseases (CVD) (Hansson & Hermansson, 2011). To date, CVD remains the major cause of mortality in the world, typically claiming a third of all deaths (Lonn et al., 2011). Inflammation and endothelial activation are the early stages in the development of atherosclerosis. During these stages, there will be over expression of cytokines [Interleukin-6 (IL-6)] and adhesion molecules [intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and e-selectin] by the ECs (ECs) (Leeuw et al., 2005). Therefore, it is suggested that IL-6, ICAM-1, VCAM-1 and e-selectin can be used as useful predictive biomarkers towards atherosclerotic progression and new targets of treatment (Poredos, 2011).

Space missions for over the past 50 years have shown that prolonged exposure of humans to microgravity may seriously affect their health (White & Averner, 2001). Among the reported health problems are cardiovascular problems related to vascular impairment which are possibly due to the modulation of the cytokine network in the ECs (Reyes et al., 1999). Modulation of the cytokine network may lead to increased inflammation and endothelial activation in the blood vessel.

ECs are sensitive to oxidative stress and gravity alterations (Buravkova et al., 2005). ECs produce active molecules such as cytokines [IL-6 and tumor necrosis factor-alpha (TNF- α)], ICAM-1, VCAM-1 and e-selectin in response to injury or oxidant stimuli (Osiecki, 2004). Increasing numbers of these active molecules will facilitate the transmigration of monocytes into the tunica intima, the key step in the initiation of