UNIVERSITI PUTRA MALAYSIA

IMMUNOREGULATION OF MICROGLIA WITH TOCOTRIENOLS

TAN SHI WEI

FPSK(m) 2012 29

IMMUNOREGULATION OF MICROGLIA WITH TOCOTRIENOLS

By

TAN SHI WEI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

FEBRUARY 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

IMMUNOREGULATION OF MICROGLIA WITH TOCOTRIENOLS

By

TAN SHI WEI

FEBRUARY 2012

Chairman : Sharmili Vidyadaran, PhD

Faculty : Faculty of Medicine and Health Sciences

Microglia, the 'brain macrophages', are the only immune cells in the central nervous system (CNS). Activated microglia are responsible for inflammatory responses and have been noted in the pathophysiology of various neurodegenerative diseases. Continuous activation of microglia resulting in chronic neuroinflammation is thought to exacerbate neuronal damage. Therefore, modulating the inflammatory responses of microglia may be the key to limiting or treating inflammatory events that occur within the CNS parenchyma. Vitamin E is well-known for its anti-inflammatory and its anti-oxidative properties. Natural vitamin E consists of eight chemically distinct compounds: α-, β-, γ- and δ-tocopherol; α-, β-, γ- and δ-tocotrienol. Interestingly, tocotrienols has shown better neuroprotective ability than tocopherols. However, to date, there is no approach in studying anti-inflammatory effects of tocotrienols on microglia responses. This study was to elucidate the possible regulatory function of tocotrienols on microglia. First, palm α-, γ- and δ-tocotrienol fragments and Tocomin®50% (a tocopherol/ tocotrienol

complex) were screened for their ability to reduce nitric oxide (NO) production by BV2 microglia (an immortalized cell line). BV2 cells were treated with tocotrienols at various concentrations (100 nM, 250 nM, 2.5 μM, 10 μM and 50 μM) for 24 hrs and stimulated with 1 μg/mL lipopolysaccharide (LPS). Highest concentration of all 4 tocotrienols fragments limited NO production by LPS-stimulated BV2 cells without affecting their cell viability, as determined by the MTS assay. Among the tocotrienols fragments tested, δ-tocotrienol reduced the NO production most by approximately 50% after 48 hrs of LPS stimulation ($p<.05$). Hence, δ-tocotrienol (3.96 μg/mL (10 μM) and 19.80 μg/mL (50 μM)) and Tocomin®50% (47.50 μg/mL and 237.50 μg/mL) were chosen for downstream experiments, by investigating their effects on i-NOS gene expression, proliferation and CD40 surface marker expression in BV2 cells. δ-tocotrienol was found not inhibiting i-NOS mRNA expression to the extent that was expected based on its ability to limit NO production by BV2 cells. Tocomin®50% on the other hand significantly inhibits i-NOS gene expression by 51% ($p<.05$), indicating that the 2 forms of vitamin E have distinct mechanisms for their ability to reduce NO. Utilising the tritiated thymidine proliferation assay, pre-treating BV2 cells with δ-tocotrienol was found to promote proliferation of both resting and LPS-stimulated microglia. On the other hand, when LPS-treated BV2 cells were post-treated with δ-tocotrienol, cells' proliferation rate was found not being affected. Apart from that, with flow cytometric immunophenotyping, both δ-tocotrienol and Tocomin®50% were discovered to reduce CD40 activation marker expression levels. 237.50 μg/mL of Tocomin®50% showed highest reduction on CD40 expression in LPS-stimulated BV2 cells by 32%. The findings from this project suggest a potential role for tocotrienols to limit the inflammatory activities of

microglia within the CNS and thus may offer a potential therapeutic agent for neurodegenerative and neuroinflammatory diseases.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

IMMUNOREGULASI MIKROGLIA DENGAN TOKOTRIENOL

Oleh

TAN SHI WEI

FEBRUARI 2012

Pengerusi : Sharmili Vidyadaran, PhD

Fakulti : Fakulti Perubatan dan Sains Kesihatan

Mikroglia, iaitu makrofaj di bahagian otak, adalah satu-satunya sel imun yang terdapat di dalam sistem saraf pusat (CNS). Mikroglia yang aktif memainkan peranan penting dalam tindakbalas keradangan dan ia juga telah dititikberat dalam patofisiologi pelbagai penyakit yang melibatkan kemerosotan neuron. Pengaktifan mikroglia yang berterusan menyebabkan keradangan neuron yang kronik dan dipercayai akan menambah buruk lagi kerosakan neuron. Oleh itu, pengawalan tindakbalas keradangan mikroglia merupakan satu kekunci untuk mengehad atau merawat keradangan yang muncul dalam persekitaran CNS. Sebagai pengetahuan, Vitamin E terkenal dengan ciri-ciri anti-radang dan anti-oksida. Vitamin E yang semulajadi terdiri daripada lapan kompaun yang berlainan secara kimia, iaitu α-, β-, γ- dan δ-tokoferol serta α-, β-, γ- dan δ-tokotrienol. Dengan menakjubnya, tokotrienol telah menunjukkan keupayaan perlindungan neurons yang lebih baik berbanding dengan tokoferol. Walau bagaimanapun, sehingga kini, masih tiada pendekatan dalam kajian tentang kesan-kesan anti-radang tokotrienol terhadap

tindakbalas mikroglia. Kajian ini bertujuan menunjukkan potensi pengawalan tokotrienols terhadap mikroglia. Pertama sekali, fragmen tokotrienol kelapa sawit α-, γ- and δ-tokotrienol dan Tocomin®50% (kompleks tokoferol/ tokotrienol) telah disaring untuk menguji keupayaan fragmen-fragmen tersebut dalam mengurangkan penghasilan nitrik oksida (NO) oleh BV2 mikroglia (satu imortalisi sel abadi). Sel BV2 telah dirawat menggunakan tokotrienol pada pelbagai kepekatan (100 nM, 250 nM, 2.5 μM, 10 μM dan 50 μM) selama 24 jam dan dirangsang menggunakan 1 μg/mL lipopolysaccharide (LPS). Keempat-empat fragmen tokotrienol pada kepekatan tertinggi dapat mengehad penghasilan NO oleh sel BV2 yang dirangsangkan menggunakan LPS tanpa menjejaskan kebolehhidupan sel BV2 tersebut, sebagaimana yang ditentukan oleh ujian MTS. Di antara fragmen-fragmen tokotrienol yang diuji, fragmen δ-tokotrienol paling banyak mengurangkan penghasilan NO, iaitu sebanyak 50% selepas 48 jam rangsangan LPS ($p<.05$). Maka, δ-tokotrienol (3.96 μg/mL (10 μM) dan 19.80 μg/mL (50 μM)) dan Tocomin®50% (47.50 μg/mL dan 237.50 μg/mL) telah dipilih untuk eksperimen yang seterusnya, dengan bertujuan menyiasat kesan mereka terhadap ekspresi gen i-NOS, proliferasi dan ekspresi penanda permukaan CD40 pada sel BV2. Didapati δ-tokotrienol tidak merencat ekspresi mRNA i-NOS seperti yang dijangka berdasarkan keupayaannya dalam mengehad penghasilan NO oleh sel BV2. Manakala, Tocomin®50% dapat merencat ekspresi gen i-NOS sel BV2 sebanyak 51% ($p<.05$), ini menunjukkan kedua-dua jenis vitamin E ini mempunyai mekanisme yang berbeza dalam keupayaan untuk mengurangkan penghasilan NO. Dengan menggunakan asai proliferasi timidina tertritium, didapati sel BV2 yang dipra-rawat menggunakan δ-tokotrienol menggalakan proliferasi kedua-dua peringkat microglia sama ada dalam

rehat atau aktif dirangsang oleh LPS. Sebaliknya, apabila mikroglia dirawat dengan δ-tokotrienol selepas rangsangan LPS, didapati rawatan tersebut tidak memberi kesan kepada kadar pembahagian sel. Di samping itu, dengan teknik sitometri aliran, didapati bahawa kedua-dua δ-tokotrienol dan Tocomin®50% mempunyai kesan pengurangan taraf ekspresi penanda pengaktifan CD40. Tocomin®50% pada 237.50 μg/mL paling banyak mengurangkan ekspresi CD40 dalam sel BV2 yang dirangsang oleh LPS, iaitu sebanyak 32%. Penemuan daripada projek ini menunjukkan tokotrienol berpotensi dalam mengehad aktiviti keradangan mikroglia dan boleh ditawarkan sebagai satu agen terapeutik untuk merawat penyakit kemerosotan neuron dan keradangan neuron.

ACKNOWLEDGEMENTS

First of all, I would like to take this opportunity to express my deepest and most sincere gratitude to my supervisor, Dr. Sharmili Vidyadaran, for her advice, guidance, encouragement and countless contribution throughout my Master degree. She inspired me all the time and really gave me a push especially when I faced failures.

A special note of appreciation also goes to Dr. Maha Abdullah as my co-supervisor for her continual supports and encouragement. My acknowledgement is also extended to all my fellow colleagues in Immunology laboratory including, Pei Shen, Yin Yin, Hema, Zul, Shins, Pooi Pooi, Yip, Rhun Yian, Wai Yee, Malini, Jap Meng, Leslie, Tong, Shin Foong, Chooi Ling, Elizabeth, Choo, Homa, Cin Dee, Steph, Cheryl, Catherine, Chou Min, Cini, Kak Aisyah, Mr. Anthony, Marshitah and Kak Zura who have made the last two years an enjoyable experience for me.

My heartfelt thanks to my family members especially my parents for their love, dedications and endless moral support. I also appreciate the motivation and support from my understanding housemates and friends to proceed for completing my degree. Next, I would like to thank the Carotech Sdn. Bhd. (Malaysia) and Assoc. Prof. Dr. Thameem Dheen from National University Singapore (NUS) for providing me the 'main ingredient': tocotrienols and BV2 microglia cell line, respectively for my study. Last but not least, I would also like to thank UPM Graduate Research Fellowship Scheme (GRF) for my personal financial support.

I certify that a Thesis Examination Committee has met on 15 February 2012 to conduct the final examination of Tan Shi Wei on her thesis entitled "Immunoregulation of Microglia with Tocotrienols" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of Thesis Examination Committee were as follows:

Rozita binti Rosli, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Daud Ahmad bin Israf Ali, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Huzwah binti Khazaai, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Durriyyah Sharifah Hasan Adli, PhD
Associate Professor
Faculty of Science
Universiti Malaya
(External Examiner)

_____
SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 June 2012

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Sharmili Vidyadaran, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Maha Abdullah, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

_____
BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

_____

TAN SHI WEI

Date: 15 February 2012

TABLE OF CONTENTS

Page