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IMMUNOREGULATION OF MICROGLIA WITH TOCOTRIENOLS

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IMMUNOREGULATION OF MICROGLIA WITH TOCOTRIENOLS

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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.



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IMMUNOREGULASI MIKROGLIA DENGAN TOKOTRIENOL

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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 μ M, 10 μ M dan 50 μ M) selama 24 jam dan dirangsang menggunakan 1 μ g/mL lipopolysaccharide (LPS). Keempat-empat fragmen tokotrienol pada kepekatan tertinggi dapat mengehad penghasilan NO oleh sel BV2 yang dirangsangkan menggunakan LPS tanpa menjelaskan 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 μ g/mL (10 μ M) dan 19.80 μ g/mL (50 μ M)) dan Tocomin[®]50% (47.50 μ g/mL dan 237.50 μ g/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 $\mu\text{g/mL}$ 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.

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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.

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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.



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Date: 15 February 2012

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
DECLARATION	xi
LIST OF TABLES	xv
LIST OF FIGURES	xvi
LIST OF ABBREVIATION	xviii
CHAPTER	
1 INTRODUCTION	1
Rationale of the Study	4
Objectives of the Research	5
2 LITERATURE REVIEW	6
2.1 Neuroinflammation	6
2.2 Microglia	8
2.2.1. Short History of Microglia	8
2.2.2. Origin and Cell Lineage of Microglia	9
2.2.3. Microglia Possess Great Heterogeneity in Distribution and Phenotype	11
2.2.4. Resting Microglia	14
2.2.5. Activated Microglia and Their Associated Molecules	15
2.2.6. Lipopolysaccharide as an Inducer for Microglia Activation	22
2.3 Role of Microglia in Neurodegenerative Diseases	23
2.3.1. Microglia in Diseases: Neuroprotective or Neurotoxic?	23
2.3.2. Incidence of Microglia in Neurodegenerative Diseases	24
2.3.3. Treatment Approaches for Neurodegenerative Diseases	29
2.4 Natural Vitamin E Family: Tocopherols and Tocotrienols	31
2.5 Tocotrienols	34
2.5.1. Structure of Tocotrienols	34
2.5.2. Natural Sources of Tocotrienols	36
2.6 Bioavailability of Tocotrienols	38
2.6.1. The Role of α -Tocopherol Transfer Protein (TTP) in Tocotrienols Bioavailability	40
2.6.2. Distribution of Tocotrienols in Different Tissues	41
2.7 Natural Neuroprotective Agent: Tocotrienols	43
2.7.1. Tocotrienols are more neuroprotective than Tocopherols	45
2.7.2. Bioavailability of Tocotrienols in Central Nervous System	46
3 MATERIALS AND METHODS	48

3.1	BV2 Cell Culture	48
3.1.1	Cell Culture	48
3.1.2	Activation	48
3.2	Vitamin E Treatment	49
3.3	Griess Assay	50
3.3.1	Principle	50
3.3.2	Cell Seeding and Activation	51
3.3.3	Griess Reaction	51
3.3.4	Establishment of NO Standard Curve	52
3.3.5	Determination of NO Production	52
3.4	MTS [3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)- 2-(4-sulfophenyl)-2H-tetrazolium] Assay	53
3.4.1	Principle	53
3.4.2	Methodology	54
3.5	Tritiated Thymidine Incorporation As say	54
3.5.1	Principle	54
3.5.2	Methodology	55
3.7	Reverse-Transcription Polymerase Chain Reaction (RT-PCR)	55
3.7.1	Principle	55
3.7.2	RNA Isolation	56
3.7.3	Reverse-Transcription	57
3.7.4	Polymerase Chain Reaction (PCR)	58
3.8	Immunophenotyping	61
3.8.1	Principle	61
3.8.2	Methodology	61
3.9	Statistical Analysis	62
4	RESULTS	63
4.1	Characterisation of BV2 Cell Growth	63
4.2	Screening Tocotrienol Fragments for Nitric Oxide-Reducing Capacity Determined Using the Griess Assay	65
4.2.1	Determination of Ideal LPS Concentration	65
4.2.2	Effects of Tocotrienols Fragments and Tocomin [®] 50% on NO Production of BV2 Cells	67
4.2.3	Effects of Tocotrienols Fragments and Tocomin [®] 50% on Cell Viability of BV2 Cells	71
4.3	Immunomodulatory Effects of δ-Tocotrienol and Tocomin [®] 50% on BV2 Cells	74
4.3.1	Determination of NO Production in δ-Tocotrienol and Tocomin [®] 50% Treated BV2 Cells	76
4.3.2	Effects of δ-Tocotrienol and Tocomin [®] 50% on Viability of BV2 Cells Determined with the MTS Assay	80

4.3.3	Effects of δ -Tocotrienol and Tocomin [®] 50% on BV2 Cell Proliferation Determined Using the Tritiated Thymidine Incorporation Assay	81
4.3.4	Effects of δ -Tocotrienols and Tocomin [®] 50% on CD40 Expression of BV2 Cells	86
5 DISCUSSION		90
5.1	BV2 Cultures	91
5.3	Immunomodulatory Effects of Tocotrienols on Microglia	92
5.3.1	Inhibitory Effects of Tocotrienols on NO Production of BV2 cells without Affecting Cell Viability	92
5.3.2	Tocotrienols Promote Proliferation of Both Resting and LPS-stimulated Microglia	100
5.3.3	δ -Tocotrienol and Tocomin [®] 50% Inhibit CD40 expression of Both Resting and LPS-stimulated Microglia	103
6 CONCLUSIONS AND FURTHER RECOMMENDATIONS		110
6.1	Conclusion	110
6.2	Recommendations for Future Studies	112
REFERENCES		114
APPENDIX I - SOLUTIONS AND REAGENTS		132
APPENDIX II- DATA SETS		146
APPENDIX III-PHAGOCYTIC ACTIVITY OF BV2 MICROGLIA		164
LIST OF PUBLICATIONS		170
BIODATA OF THE STUDENT		174