UNIVERSITI PUTRA MALAYSIA

IMMUNOMODULATORY ACTION OF NEW CURCUMIN ANALOGUE [2,6-BIS(2,5-DIMETHOXYBENZYLIDENE) CYCLOHEXANONE] ON MAJOR INFLAMMATORY MEDIATOR EXPRESSION IN ACTIVATED MACROPHAGE AND SYNOVIAL FIBROBLAST CELLS

LEE KA HENG

FBSB 2012 34

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By

LEE KA HENG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,

in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

April 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

IMMUNOMODULATORY ACTION OF NEW CURCUMIN ANALOGUE [2,6-BIS(2,5-DIMETHOXYBENZYLIDENE) CYCLOHEXANONE] ON MAJOR INFLAMMATORY MEDIATOR EXPRESSION IN ACTIVATED MACROPHAGE AND SYNOVIAL FIBROBLAST CELLS

By

LEE KA HENG

April 2012

Chairman: Syahida binti Ahmad, PhD

Faculty: Biotechnology and Biomolecular Sciences

Rheumatoid arthritis is a chronic inflammatory disease characterized by abnormal immune phenomena involving macrophage and synovial fibroblast resulting in progressive joints destruction. In this study, a series of newly synthesized curcumin diarylpentanoids were screened for their anti-inflammatory properties by evaluating the nitric oxide (NO) inhibitory activity upon activated macrophages *in vitro*. The preliminary screening results have shown that curcumin-like BDMC33 [2,6-bis(2,5-dimethoxybenzylidene) cyclohexanone] exerted improved nitric oxide inhibitory activity (IC_{50} value = 13.66 ± 0.61 μM) as compared to curcumin (IC_{50} value = 27.13 ± 5.58). Therefore, we further investigated the immunomodulatory action of BDMC 33 on two cellular systems which are IFN-γ/LPS-stimulated macrophages (RAW 264.7) and PMA-stimulated synovial fibroblast (HIG-82). BDMC33 was found to inhibit the NO and PGE_2 production through down-regulation of iNOS and COX-2 expression in activated macrophages without altering its enzyme activities, respectively. In addition, effect of BDMC33 was observed to inhibit production of TNF-α and IL-1β at the protein and gene expression level. Further study demonstrated that the inhibitory activities of BDMC33 was mediated by interfering

in NF-κB signal transduction pathway, includes inhibition of NF-κB DNA binding activities and p65 NF-κB nuclear translocation as well as prevent rapid phosphosrylation and degradation of IκB subunit. Moreover, DNA binding activities of AP-1 also appear to be inhibited by BDMC33 through attenuation of ERK1/2 and JNK1/2 phosphorylation. On the other hand, BDMC33 significantly inhibited the MMP-9 activities as well as collagenase activities via suppression of MMP-1 expression upon activated synovial fibroblast. Moreover, BDMC33 strongly suppressed *MMP-3* gene expression as well as inhibited *COX-2* and *IL-6* inflammatory gene expression in activated synovial fibroblast. The underlying mechanism of BDMC33 on synovial fibroblast was also mediated via NF-κB signaling pathway; as p65 NF-κB nuclear translocation and NF-κB DNA binding activity are being attenuated. Collectively, the experimental data suggested that the immunomodulatory action of BDMC33 is attributed through interference in inflammatory mediator expression of both macrophages and synovial fibroblast, which could lead to its possibility into future evaluation of the *in vivo* or pre-clinical study.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

TINDAKAN IMMUNOMODULATOR DARI KUKURMIN ANALOG BARU [2,6-BIS(2,5-DIMETHOXIBENZILIDINE) SIKLOHEXANON] TERHADAP EKSPRESI INFLAMASI MEDIATOR UTAMA DALAM SEL MAKROFAJ DAN FIBROBLAST SINOVIAL YANG DIRANSANGI

By

LEE KA HENG

April 2012

Pengerusi: Syahida binti Ahmad, PhD

Fakulti: Bioteknologi dan Sains Biomolekul

Artritis Reumatoid adalah penyakit keradangan kronik yang dicirikan oleh fenomena imun yang abnormal melibatkan makrofaj dan sel fibroblas sinovial yang mengakibatkan kerosakan sendi yang progresif. Dalam kajian ini, satu siri diarylpentanoids kurkumin disaring secara *in vitro* bagi sifat anti-inflamasi dengan menilai aktiviti perencatan oksida nitrik (NO) pada makrofaj yang diaktifkan. Hasil pemeriksaaan awal mununjukkan bahawa BDMC33 [2,6-bis(2,5-dimethoxibenzilidine) siklohexanon] iaitu salah satu kurkumin analog telah mempamerkan peningkatan aktiviti penghambatan oksida nitrik (nilai IC_{50} =13.66 ± 0.61 μM) berbanding dengan kurkumin (nilai IC_{50} == 27.13 ± 5.58 μM). Oleh itu, penyelidikan dilanjutkan bagi mengkaji sifat-sifat anti-rematik dari BDMC33 serta mengenalpastikan mekanisme yang mungkin ditindaki oleh BDMC33 pada dua sistem sel, iaitu sel makrofaj (RAW 264.7) yang diaktifkan oleh IFN-γ /LPS dan sel fibroblas sinovial (HIG-82) yang diaktifkan oleh PMA. BDMC33 didapati menghalang penghasilan NO dan PGE_2 melalui perencatan iNOS dan COX-2 sintesis tanpa mengubah aktiviti enzim iNOS dan COX-2 pada sel makrofaj yang diaktifkan masing-masing. Tambahan pula, pengaruh BDMC33 diamati untuk menghalang

penghasilan TNF-α dan IL-1β pada tahap protein dan expresi gen. Lanjutan kajian menunjukkan bahawa kegiatan rencat BDMC33 diperantarakan oleh gangguan dalam pusat isyarat transduksi NF-κB, termasuklah perencatan kegiatan pengikatan DNA NF-κB dan p65 NF-κB translokasi nuklear serta mencegah pantas fosforilasi dan degradasi subunit IκB. Selain itu, aktiviti kegiatan pengikatan DNA AP-1 juga dihambat oleh BDMC33, kemungkinan melalui perencatan ERK1/2 dan JNK1/2 fosforilasi. Di sisi lain, BDMC33 secara signifikasi merencat aktiviti MMP-9 serta aktiviti kolagenase melalui penurunan ekspresi MMP-1 pada sel fibroblas sinovial yang diaktifkan. Selain itu, BDMC33 kuat menekan ekspresi gen MMP-3 serta menghalangkan ekspresi gen inflamasi COX-2 dan IL-6 meskipun dalam tahap yang sederhana pada sel fibroblas sinovial yang diaktifkan. Mekanisme yang mendasari BDMC33 pada sel fibroblas sinovial juga dimediasi melalui isyarat NF-κB: seperti p65 NF-κB translokasi nuklear dan kegiatan pengikatan DNA NF-κB. Secara keseluruhan, data kajian menyarankan bahawa tindakan immunomodulator BDMC33 yang disebabkan gangguan dalam ekpresi pathogen mediator utama dari kedua-dua sel makrofaj dan fibroblas sinovial, membawa kemungkinan bagi penglibatan dalam kajian in vivo atau pra-klinikal pada masa depan.

ACKNOWLEDGEMENT

First and foremost, I would like to express my most sincere gratitude and deep appreciation to my supervisors, Dr. Syahida Ahmad for her invaluable advices guidance, knowledge and encouragement throughout the course of this study.

I would like to extend my appreciation to my co-supervisors, Professor Dr. Nordin Haji Lajis, Associate Professor Dr. Faridah Abas, and Dr. Noorjahan Banu Mohamed Alitheen for their generous input, constructive suggestion, advices and necessary support.

Subsequently, I would like to express my sincere gratitude to my colleagues, all the staff and friends from Faculty Biotechnology and Biomolecular Sciences, Institute Bioscience and Faculty Medicine and Health Sciences for their kind, excellent and constant technical assistance.

Last but not least, I would like to express my deepest blessing and gratitude to my beloved family for their love, sacrifice, moral support and kind patience.

I certify that a Thesis Examination Committee has met on 27th April 2012 to conduct the final examination of Lee Ka Heng on his PhD thesis entitled "Immunomodulatory action of new curcumin analogue [2,6-bis(2,5-dimethoxybenzylidene) cyclohexanone] on major inflammatory mediator expression in activated macrophage and synovial fibroblast cells" 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 degree of Doctor of Philosophy.

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DECLARATION

I declare that the thesis is my original work except for the synthesis of tested compounds, 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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LEE KA HENG

Date: 27th April 2012

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