UNIVERSITI PUTRA MALAYSIA

ANTINOCICEPTIVE PROFILE OF A SYNTHETIC CURCUMINOID DERIVATIVE 2,6-BIS-(4-HYDROXY-3-METHOXY-BENZILIDINE)-CYCLOHEXANONE IN MURINE MODEL OF INDUCED-NOCICEPTION

LEE MING TATT

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By

LEE MING TATT

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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

ANTINOCICEPTIVE PROFILE OF A SYNTHETIC CURCUMINOID DERIVATIVE 2,6-BIS-(4-HYDROXY-3-METHOXY-BENZILIDINE) CYCLOHEXANONE IN MURINE MODEL OF INDUCED-NOCICEPTION

By

LEE MING TATT

February 2013

Chairman: Professor Mohd. Roslan Sulaiman, PhD

Faculty: Faculty of Medicine and Health Sciences

Introduction: The present study investigated the antinociceptive potential of a synthetic curcuminoid derivative, 2,6-bis-4-(hydroxyl-3-methoxybenzilidine)-cyclohexanone, or BHMC in pain-induced models in mice. **Problem statement**: Pain is a major symptom of various diseases that persists to produce severe physical and psychological distress for many patients. However, current treatment for pain produced undesirable adverse effects thus limited their use. This has led to a search for new pharmacologically potent antinociceptive compounds with minimum or no adverse effects. **Objective**: The present study aimed to investigate the systemic, peripheral and supraspinal antinociceptive potential of BHMC in pain models in mice. The research project also aimed to further investigate the possible mechanism of action in systemic, peripheral and supraspinal antinociceptive effect of BHMC. **Methodology**: Systemic antinociceptive effect of

BHMC was determined using acetic acid-induced abdominal constriction test, formalin induced-paw licking test and hotplate test. The systemic effect of BHMC was also evaluated via chronic constriction injury-induced neuropathic pain in mice. Peripheral and supraspinal antinociceptive effect of BHMC was evaluated via carrageenan-induced hyperalgesia test and thermal-induced nociception test, respectively. **Results and discussion**: It was demonstrated that systemic treatment of BHMC exhibited dose-dependent inhibitory effect in chemical- and thermal-induced nociception. Further investigation on the involvement of descending modulatory pathway in systemic antinociceptive effect of BHMC showed that BHMC selectively activated κ-opioid, A_1-adenosine, D_2-dopamine, M-acetylcholine, $GABA_B$, α_2-noradrenaline and 5-HT_{A1} receptors. Activation of these inhibitory receptors triggered the efflux of potassium ion from nociceptive neuron, leading to neuronal hyperpolarisation. Hyperpolarisation of nociceptive neuron via nitric oxide (NO) independant cyclic guanosine monophosphate (cGMP) induced potassium (K^+) channel opening was shown as one of the possible antinociceptive mechanism of systemic antinociceptive action of BHMC. Dose-dependant inhibitory effect of BHMC was also shown in CCI-induced hyperalgesia test in mice, which indicated a similar mechanism of action in BHMC-induced antinociception. It was also demonstrated that peripheral and supraspinal administration of BHMC produced dose-dependent antinociceptive effect, with peripheral antinociceptive effect of BHMC was via μ-,κ-opioid receptor and NO-independent cGMP/protein kinase G (PKG)/K^+ channel activation; whereas supraspinal analgesic effect of BHMC was through κ-opioid/NO/cGMP/PKG/K^+ channel activation. **Conclusion**: The present study demonstrated that BHMC possessed potential systemic, peripheral and supraspinal antinociceptive effect

through mechanisms that caused the activation of K+ channels leading to neuronal membrane hyperpolarisation.

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

KAJIAN KESAN ANTINOSISEPTIF 2,6-BIS-4-(HIDROKSI-3-METHOXYBENZYLIDINE)-CYCLOHEXANONE TERHADAP MODEL NOSISEPSI MENCIT

Oleh

LEE MING TATT

Februari 2012

Pengerusi : Profesor Mohd. Roslan Sulaiman, PhD

Fakulti: Fakulti Perubatan dan Sains Kesihatan

Pengenalan: Projek ini telah dijalankan untuk mengkaji kesan antinosiseptif satu sebatian sintetik analog kurkumin, 2,6-bis-4-(hidroksi-3-methoxy-benzilidine)-cyclohexanone atau BHMC ke atas model mencit yang diaruh kesakitan. **Penyataan masalah**: Sakit merupakan simtom utama bagi kebanyakan penyakit yang sekiranya berkekalan boleh menyebabkan tekanan fizikal dan psikologi yang serius kepada pesakit. Walaubagaimana pun, rawatan terkini untuk sakit menghasilkan kesan-kesan sampingan tidak dikehendaki yang mengehadkan penggunaannya. Oleh sebab itu, kajian bagi mencari sebatian antinosiseptif yang berkesan secara farmakologikal dengan kesan sampingan yang minimal atau langsung tiada kesan sampingan. **Objektif**: Tujuan utama projek ini adalah untuk mengkaji potensi BHMC sebagai sebatian yang mempunyai kesan antinosiseptif secara sistemik, periferi dan supraspinal dengan menggunakan model model kajian kesakitan dalam mencit.. Projek ini juga bertujuan untuk mengkaji mekanisme

tindakbalas BHMC yang berkemungkinan dalam memberi kesan antinosiseptif secara sistemik, periferi dan supraspinal. **Metodologi kajian**: Kajian kesan antinosiseptif BHMC dijalankan dengan menggunakan ujian kekecutan otot abdomen aruhan asid asetik, ujian penjilatan tapak kaki aruhan formalin dan ujian plat panas. Efikasi kesan sistemik BHMC juga dikaji menggunakan model sakit neurogenik dalam mencit. Kesan antinosiseptif periferi dan supraspinal BHMC dinilai melalui ujian hiperalgesia aruhan carrageenan dan ujian nosisepsi aruhan haba. **Keputusan dan perbincangan**: BHMC menunjukkan aktiviti antinosiseptif sistemik berkadaran dos ke atas ujian pengecutan otot abdomen aruhan asid asetik, ujian penjilatan tapak kaki aruhan formalin dan ujian plat panas. Kajian lanjutan menunjukkan BHMC mengaktifkan reseptor κ-opioid, A_1-adenosine, D_2-dopamine, M-acetylcholine, $GABA_B$, α_2-noradrenaline dan 5-HT_{A1}. Tambahan pula, mekanisme antinosiseptif sistemik BHMC adalah melalui hiperpolarisasi saraf nosiseptif oleh pengaktifan saluran kalium (K^+) melalui siklik guanosin monofosfat (cGMP) tanpa nitrik oxida (NO). Mekanisme yang sama juga ditunjukkan dalam kesan antinosiseptif BHMC terhadap model sakit neuropatik dalam mencit. Kajian antinosiseptif periferi and supraspinal BHMC menunjukkan bahawa BHMC memberi kesan perencatan berkadaran dos ke atas ujian hiperalgesia induksi carrageenan dan ujian nosisepsi aruhan haba, masing masing. Kajian mekanisme antinosiseptif periperi menunjukkan bahawa BHMC mengaktifkan saluran K^+ melalui cGMP/protein kinase G (PKG) tanpa NO dan reseptor μ- serta κ-opioid. Kajian antinosiseptif secara supraspinal BHMC menunjukkan bahawa memberi kesan perencatan berkadaran dos ke atas ujian ujian nosisepsi induksi haba dan mekanisme antinosiseptif secara supraspinal adalah melalui pengaktifan saluran K^+ melalui NO/cGMP/PKG dan reseptor κ-

opioid. **Kesimpulan**: Projek ini membuktikan aktiviti antinosiseptif BHMC berlaku melalui sistemik, periferi dan supraspinal menerusi mekanisma yang membawa kepada pengaktifan saluran K^+ yang membawa kepada hiperpolarisasi sel membran saraf.

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I certify that a Thesis Examination Committee has met on 8th of February 2013 to conduct the final examination of Lee Ming Tatt on his thesis entitled "Antinociceptive Profile of a Synthetic Curcuminoid Derivative, 2,6 bis-(4-hydroxy-3-methoxy-benzilidine)-cyclohexanone on Murine Model of Induced-nociception" 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 Doctor of Philosophy.

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

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Date:

DECLARATION

I declare that the thesis is my original work except for 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 MING TATT

Date: 8 February 2013

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