UNIVERSITI PUTRA MALAYSIA

ANTI-INFLAMMATORY PROPERTIES OF 3-(2,5-DIMETHOXYPHENYL)-1-(5-METHYLFURAN-2-YL) PROP-2-EN-1-ONE (L31) IN LUNG EPITHELIAL CELLS AND ON AIRWAY INFLAMMATION IN A MURINE MODEL OF ASTHMA

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FPSK(m) 2014 52

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By

REVATHEE A/P RAJAJENDRAM

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the requirement for the Degree of Master of Science

February 2014

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

ANTI-INFLAMMATORY PROPERTIES OF 3-(2,5-DIMETHOXYPHENYL)-1-(5-METHYLFURAN-2-YL)PROP-2-EN-1-ONE (L 31) IN LUNG EPITHELIAL CELLS AND ON AIRWAY INFLAMMATION IN A MURINE MODEL OF ASTHMA

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February 2014

Chairman: Professor Daud Ahmad Israf Ali, PhD
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Diverse heterocyclic compound's production involved chalcones. Many studies have proven that chalcone derivative compound possess assorted biological activities such as antimicrobial, anti-inflammatory, analgesic, antiplatelet, antiulcerative, antimalarial, anticancer and antiviral properties. Cardamonin's analogues 3-(2,5-dimethoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (L31) was synthesized. Cell viability was resolved by conducting MTT cytotoxicity assay. L31 has a firm suppression effect upon eotaxin, MCP-1 and RANTES but did not inhibit the secretion of chemokines GRO-α and IL-8. The molecular target of L31 on several major proinflammatory pathways was furtherdissected. The results from western blots reveal that L31 targets the NF-kB but not the MAPK pathway. These findings confirm the selectivity and specificityof L31. Dose-response studies were conducted on female Balb/c mice in which doses (0.2, 2, 20 and 100 mg/kg) of L31 were administered intraperitoneally to mice following sensitization and aerosolized doses of ovalbumin (OVA). Mice were then subjected to methacholine challenge with an ultrasonic nebulizer and airway hyperresponsiveness was determined by comparison of enhanced pause (Penh) responses. Whole-body plethysmography system (Buxco) was used to measure the Penh value. Analysis of bronchoalveolar lavage (BALF) showed that all doses of L31 caused significant reduction in airway eosinophilia, tissue inflammatory scores and goblet cell metaplasia. L31 suppressed the secretion of eotaxin, RANTES, interleukin (IL) 4, IL 5 and IL 13. L31 also inhibits the gene expression of the all the mediator except for IL13. Collectively, these findings are vital in making decisions for further development of L31 compounds into anti-inflammatory and anti-asthmatic drug. The ultimate aim would be to enter clinical trials since the current therapy involves administration of combinations of steroids and β-agonists which in long term would mask untreated inflammation and lead to a higher risk of adverse outcomes.

Abstrak tesis yand dikemukakan kepada Senat Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk ijazah Master Sains

PENGARUH ANTI INFLAMATORI 3-(2,5-DIMETHOXYPHENYL)-1-(5-METHYLFURAN-2-YL) PROP-2-EN-1-ONE DI SEL EPITHELIUM DAN SALUR PERNAFASAN MURIN TERHADAP MODEL KAJIAN ASMA.

Oleh

REVATHEE A/P RAJAJENDRAM

Februari 2014

Pengerusi: Professor Daud Ahmad Israf Ali, PhD
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Chalkon merupakan sebuah sebatian yang terkenal untuk mensintesiskan pelbagai jenis heterosikal.Sebatian yang mempunyai tulang belakang chalkon dipercayai mempunyai pelbagai aktiviti biologi seperti anti-mikrorganisma, anti-keradangan, ubat bius, anti-platelet, anti-ulcer, anti- malaria, anti- kanser dan anti-virus. Analog Cardamonin 3-(2,5-dimethoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (L31) telah disintesis. Kebolehan sel untuk hidup telah ditetapkan melalui kajian MTT.L31 mampu menyebabkan kerencatan terhadap eotaxin, MCP-1 dan RANTES tetapi tidak dapat merencatkan perembesan GRO-α dan IL-8. L31 mentargetkan beberapa jenis pengantara pro-inflamasi. Keputusan blot mengumumkan bahawa L31 mentargetkan NF-kB tetapi bukan pada aliran MAPK. Ini menunjukkan L31 adalah khusus dan memilih. Kajian dos respons dilakukan dengan menggunakan mencit Balb/c betina pada dos L31(0.2, 2, 20 dan 100 mg/kg).L31 diberikan secara intraperitoneal pada mencit selepas empat minggu immunisasi dengan ovalbumin (OVA) secara parenteral and aerosol. Sehari selepas cabaran OVA yang terakhir, mencit dicabar dengan peningkatan dos aerosol methacholine dan AHR diukur melalui respon enhanced pause (Penh) dengan menggunakan sistem whole body plethysmograph. Analisa cecair bronchoalveolar lavage (BALF) menunjukkan bahawa L31 menyebabkan pengurangan eosinofil, keradangan tisu and goblet sel L31 menghadkan perembesan eotaxin, RANTES, interlukin (IL) 4, IL 5 and IL 13 dan pengexpressan gene kecuali untuk IL 13. Secara keseluruhannya, keputusan pencarian ini adalah penting untuk membuat keputusan agar L31 dapat dijadikan sebagai ubat anti-keradangan dan anti-asma. Objectif unggul adalah untuk meneroka percubaan klinikal kerana terapi terkini iaitu kombinasi steroid dan β-agonists akan menyebabkan kesan sampingan yang negatif.

ACKNOWLEDGEMENTS

I would like to express my heartfeltgratitude to my project supervisor; Professor Dr. Daud Ahmad Israf Ali for his continues guidance, supervision and encouragement throughout the course of this project. Hisinspiring ideas and invaluable advice throughout my entire research has enhanced my growth as a student, a researcher and a scientist want to be.His generosity in continuously sharing his knowledge, time and patience throughout my research, thesis writing and presentation was very much appreciated. Under his expert guidance and encouragement, I had undergone a very good academic training and also learnt so much from him.

I would like to give special thanks to my project co-supervisors, Professor Dr. Mohd Roslan Sulaiman and Dr Kim Min Kyu for their concern and advice. Their generous input, constructive criticism, and invaluable advice in my project have helped me to complete this project successfully. I also would like to extend my sincere thanksto Dr Mohamad Nadeem Akhtarfor synthesizing the compound and providing it to me throughout the project timeline.

I would like to acknowledgeDr Tham Chau Ling for her constructive advice and tremendous help in my research project. She gave me emotional support and has been my inspiration when I face problems in the process of completing this project. My sincere appreciation also goes to my fellow laboratory colleagues and friends.Thanks a lot for sacrificing their time, giving opinion and sharing information throughout this course. I would also like to express my gratitude sharing their wealth of technical knowledge and emotional support.

I would like to acknowledge all the staffs from Cell Signaling Laboratory and Physiology Laboratory for their excellent and kind assistance in technical issues. I want to thank all the staffs in Cell Signalling Laboratory, Ms. Norazren Ismail, Ms. Norasyikin Salim and Mr. Zulkhairi Zainol for their technical assistance. Besides that, my deep appreciation goes to all the staffs in Animal House, Physiology Laboratory and Histopathology Laboratory for their warm-hearted help and cooperation.

Finally, I would like to thank my dearest parents and family members for their sacrifice, moral support and constant love all this time. Their immeasurable supports were always been there whenever I need help.

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

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LIST OF ABBREVIATIONS

AHR	airway hyperresponsiveness
ANOVA	one-way analysis of variance
APCs	antigen-presenting cells
ATP	adenosine triphosphate
BALF	bronchoalveolar lavage fluid
BCA	bicinchoninic acid
BSA	bovine serum albumin
CD14	monocyte differentiation antigen CD14
COX-2	cyclooxygenase-2
Dex	dexamethasone
DMEM	Dulbecco's Modified Eagle Medium
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
ERK	extracellular signal-regulated kinase
FBS	fetal bovine serum
FDA	Food and Drug Administration
GADPH	glyceraldehyde-3-Phosphate Dehydrogenase
HRP	horseradish peroxidase
IC50	half maximal inhibitory concentration
ICAM-1	intercellular adhesion molecule type 1
IFN-γ	interferon-γ

IgE	immunoglobulin E
IL	interleukin
JNK	c-Jun N-terminal kinases
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemotactic protein-1
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NF-κB	nuclear factor-kappaB
OVA	ovalbumin
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PD 98059	2´-amino-3´-methoxyflavone
PVDF	polyvinylidene fluoride
RNA	ribonucleic acid
RPMI	Roswell Park Memorial Institute
RT-PCR	reverse transcription-polymerase chain reaction
SB 203580	4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4- pyridyl)imidazole
SDS	sodium dodecyl sulphate
SEM	standard error of the mean
SP 600125	anthra [1,9-cd]pyrazol-6(2H)-one
TBE	tris/Borate/EDTA
TBST	tris buffered saline-tween 20
TEMED	tetramethylethylenediamine
TFIIB	transcription factor II B
Th	T helper
TLRs	toll-like receptors

TNF-α	tumor necrosis factor – α
UV	ultraviolet
VCAM-1	vascular endothelial cell adhesion molecule type 1

LIST OF ANNOTATIONS

α	alpha
β	beta
ºC	degree celcius
γ	gamma
g	gram
kDa	kilodalton
kg	kilogram
<	lesser than
L	litre
µ	micro-
µg	microgram
µL	microlitre
µM	micromolar
mg	milligram
mL	milliliter
mM	millimolar
M	molar (mol/litre)
n	nano-
p	pico-
±	plus and/or minus
U	un

CHAPTER 1

INTRODUCTION

Asthma is an immunologically mediated chronic allergic disease of lower airways due to type I hypersensitivity to common allergens that are harmless to normal people such as pollen, mite dust, house dust, animal dander and food (Choi, J. et al., 2009). It is always identified by airway hyperresponsiveness (AHR). Besides that airway edema and mucus hypersecretion are also associated with asthma(Moon et al., 2008).Type 2 T helper (T_H2) cells are being important in asthma as they secrete cytokines such as interleukin 4 (IL 4), IL 5 and IL 13 that mediate the inflammatory responses (Iwamura et al., 2010).

There is a global increase in the incidence, mortality and morbidity caused by asthma even though there is an expanding repertoire of medications available for both acute and chronic asthma treatment and management. According to World Health Organization (WHO), there are about 300 million people are suffering from asthma and this number might increase to an additional 100 million people by 2025 (Bousquet et al., 2005).In Malaysia, asthma is the third highest ranking chronic disease based on Third National Health and Morbidity Survey (NHMS III, 2006). Asthma affects about 10-13% of total population in Malaysia (Tee, 2005). It not only influences the quality of life and restricts the physical, emotional and social activities of asthmatic patients; it is also an economic burden in terms of expensive health care costs and low productivity.

Current treatments for asthmatic patients shown in Table 1.1 are the inhaled corticosteroids and long acting β_2-agonists. Inhaled corticosteroids and β_2-agonsists have systemic side effects (such as fragility of skin capillary, dermal thinning, adrenal suppression, cataracts and osteoporosis) and local side effects (such as dysphonia, cough and oropharyngeal candidiasis)(Barnes, 2005). It is necessary to develop new classes of drugs that can be taken orally and that are more effective than existing medications. It should be specifically without side effect and effective in poorly controlled asthma.

Bronchodilators	Anti-inflammatory therapies
Inhaled short acting $\beta_2$agonists: sabutamol and terbutaline	Inhaled corticosteroids: budesonide, fluticasone propionate, beclomethasone dipropionate and mometasone
Inhaled long acting $\beta_2$agonists: salmeterol and formoterol	Antileukotrienes: montelukast, pranlukast and zafirlukast
Inhaled anticholinergics: ipratropium bromide and tiotropium bromide	Cromones: sodium cromoglycate and nedocromilsodium
Theophylline: slow release theophylline and aminophylline	Anti-immunoglobulin E: omalizumab

Table 1: Current therapies for asthma. (Barnes, 2004).

Recently, much interest has gone into developing a more effective and specific therapy for asthma. Another crucial process in the progression of asthma disease is the recruitment of inflammatory cells into the airways by chemokines such as RANTES, MCP-1 and MIP-1α (Conti andDiGioacchino, 2001).Excessive and persistentinflammation causes various humandiseases and also regulates the pathophysiological state of diseases. Chemokines and its receptors are considered promising target for the regulation ofleukocyte infiltration in inflammatory and immune diseases. The characterizationof chemokines has revealed the molecular mechanisms underlying specific leukocyte subset infiltration into inflammatory tissues. During infection, inflammation, tissue injury, and tumors, the chemokines regulate the enrollment of effector leucocytes (Moser & Willimann, 2004). As chemokines therefore is an important trigger for recruitment of immune cells to the lung during the asthamatic attack.

A recent study reported that several natural chalcone derivatives have been found to exhibit potential anti-inflammatory property in allergic asthma. Recently, Iwamura and his colleagues (2010) had proven naringenin chalcone (NGC) which is extracted from tomato skin exhibited anti-asthmatic effect. NGC attenuated the extent of airway hyper responsiveness (AHR); reduced the number of eosinophils, neutrophils, lymphocytes, and macrophages infiltrating the airways; decreased the level of mucus hypersecretion; and lowered the production of IL-4, IL-5 and IL-13 by splenic CD4 T cells.

3-(2,5-dimethoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (L31) is a synthetic chalcone analogue. It is a cardamonin's analogue. Chalcones which belong to the flavonoid family are made up of two aromatic rings(Nowakowska, 2007). Studies show that chalcones have anti cancer, anti inflammatory, anti microbial and anti oxidant properties. Several chalcone derivatives have also been proven to suppress airway inflammation and AHR in allergic asthma (Iwamura et al., 2010).

The findings of Iwamura (2010) indicate that chalcone was a potential anti-inflammatory candidate in cellular models of inflammation. Therefore, in order to justify the anti-inflammatory effects of L31 in models of inflammation, L31 was introduced into the epithelial cell and murine model of acute asthma to further examine the potential of L31 to be developed into an anti-inflammatory agent in the future.

1.1 Objective

1.1.1 General objective

To determine the anti-inflammatory properties of L31 in lung epithelial cells and asthma.

1.1.2 Specific objectives

i. To determine the effects of L31 on chemokines secretion in TNF-α induced A549 human lung epithelial cells

ii. To determine the potential suppressive effect of L31 upon airway hyperresponsiveness (AHR) in asthamatic mice.

iii. To determine the effects of L31 on gene and protein expressions of chemokines in lung tissue of asthmatic mice.

iv. To determine the potential suppressive effect of L31 upon pulmonary inflammation.

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