Investigation of the immunostimulatory effects of some New Zealand honeys and characterization of an active component

A Thesis submitted in partial fulfilment of the requirements for the degree of

Master of Technology

in

FOOD TECHNOLOGY

at Massey University, Albany

New Zealand

Swapna Gannabathula

June 2010

Abstract

Medicinal use of honey has re-emerged recently indicating that honey accelerates wound healing activity. Honey has been shown to stimulate TNF-α production from monocytes and macrophages which is apparently correlated with a high molecular weight fraction, and not lipopolysaccharide (LPS, an immunostimulatory endotoxin) levels. Cytokine production by honey has been attributed to the endotoxin content. The aim of this study was to investigate the ability of Comvita sourced honeys to elicit a TNF-α cytokine response from acute monocytic leukemia (THP-1) cells as well as identify the responsible component.

Five honey samples were used together with sugar and methylglyoxal controls. The samples were incubated with THP-1 cells, with and without LPS. After incubation, the cell culture supernatants were collected and TNF-α was measured by the enzyme-linked immune sorbent assay (ELISA). The most active honey samples were further heat-treated to remove enzyme/protein/peptide-like stimulation; the samples were treated with polymixin B (PmB) to remove LPS-like stimulation and not protein fraction. The samples were then filtered by molecular weight centrifugal filters to separate constituents according to their size and the fractions were re-analysed.

All five honey samples in the absence of LPS stimulated TNF-α release from THP-1 cells, whereas untreated, sugar- and methylglyoxal-treated cells did not. The cytokine production was partially inhibited by heating, but mostly by PmB. In the filtered honey samples, the activity was observed in the >30 kDa fraction. These results suggest that the activity may be associated with one or more components which are partially heat-labile, LPS-like stimulated with a high molecular weight.

Further, honey samples were analyzed for the concentration of LPS present. The tests revealed that the cytokine stimulation was higher than would be expected from the concentration of LPS present in the honey. The possibility that this component was a plant-derived β-glucan, which is known to have LPS-like activity and can interfere with detection of LPS in the LAL assay, was investigated. Subsequent analyses confirmed the presence of arabinogalactan, a large complex carbohydrate.

The data presented in this study suggests that arabinogalactans in honey may stimulate inflammatory responses and the release of cytokines that are crucial in regulating wound-healing. This heralds a significant advancement in the usage and understanding of medicinal honey.

Acknowledgements

I would particularly like to take this opportunity to convey my sincere gratitude and appreciation to the following people and institutions for their advice, support, help and encouragement throughout the tenure of my study.

First of all, I thank God for guarding safety, good health and showing me the right way throughout my life.

Sincere and appreciate acknowledgements to my supervisor, Dr. Doug Rosendale, for offering me this opportunity, his advice, and support throughout the course of this study. His constant inspiration, enthusiasm, strong motivation and guidance in the subject area were appreciated. Not only did he give me prompt advice and answers to my questions, but he also enabled me to be able to think about aspect of the work from different angles.

I would like to express equally important appreciative acknowledgments to my supervisors, Professor Margot Skinner and Dr. Tony Mutukumira, for their continuous support, guidance, encouragement, and kind co-operation throughout my study. I am very much thankful to Dr.Tony Mutukumira for giving me the opportunity to study at the Institute of Food, Nutrition and Human Health, Massey University. A special thanks to Professor. Margot Skinner for her kind helps as I wrote my thesis.

Thanks to Dr Ralf Schlothauer and Dr Jonathan Stephens, from Comvita New Zealand Limited, for creating and directing the programme, for providing me honey samples and their support, guidance, enthusiasm, strong motivation, and proof reading of my thesis.

I am thankful to Dr. Jeff Greenwood, Edward Walker and Judie Farr, for their kind help, assistance in learning cell culture techniques and ELISAs.

I would like to express my thanks to Dr. Paul Loong from ESR and Dr. Ian Sims from IRL for their work outlined in Chapter 5.

I would like to acknowledge Graham Fletcher, Graeme Summers and Cristina Cruz for their valuable guidance, friendly attitude, answering my silly questions, suggesting to me the right path and support not only throughout this study but also outside of it.

My personal thanks to, Jill Chantarachoti, Sravani Gupta, Jessicah Win, Sabrina Tian, Sean Chen and Andrew Boey, for their kind help, co-operation and patience and, it was great to share daily moments with them and Monday morning teas.

Words cannot describe my heartiest gratitude towards my husband Subrahmanyeswarlu Gannabathula. This thesis would not have been possible without the advice, love, companionship, support, encouragement and guidance from him. His lively, optimism, faithfulness and his passion for study, understanding and patiently kept telling me it could be done, and whose hard work can be seen on every page. Subbu you are one in a million and came into my life as light, happiness and without you I could not have been what I am and where I am now.

Comvita New Zealand Limited and the New Zealand Foundation for Research Science and Technology for providing me with a scholarship, research funding, arranging research facilities, and conference travel grant; the New Zealand Institute for Plant & Food Research Limited for providing research facilities; the Institute of Food, Nutrition and Human Health, Massey University for providing the Master candidature registration and travel grant.

Last but not least I am really indebted to my parents Jhansi Rani Maraoni (Mom) Ramdas Maraoni (Dad) and my daughters Yashaswini Gannabathula and Harika Gannabathula for their love, companionship, help, and continuous support through the whole process of this study. The words of encouragement, moral support and valuable guidance from my parents made it possible to complete my study.

To my Husband Subrahmanyeswarlu, and daughters, Yashaswini and Harika
May you live a long and happy life,
With all my love

List of Contents

List of Figures

List of Tables

List of Abbreviations

NH_4OH	Ammonium hydroxide
ATCC	American Type Culture Collection
ANOVA	Analysis of variance
AG	Arabinogalactan
AGP	Arabinogalactan-protein
AH	Artificial honey
BSA	Bovine serum albumin
$(CH_3)_2SO$	Camptothecin
CO_2	Carbon dioxide
^oC	Degrees Celsius
DMSO	Dimethylsulfoxide
KH_2HPO_4	Dipotassium hydrogen orthophosphate
Na_2HPO_4	Disodium hydrogen orthophosphate anhydrous
ECM	Extra cellular matrix
ESR	Environmental Science Research
ELISA	Enzyme-linked immune sorbent assay
FBS	Fetal bovine serum
GC-MS	Gas chromatography-mass spectrometry
g	Gram or Acceleration due to gravity
>	Greater than
HPAEC	High-performance anion-exchange chromatography
h	Hour
THP-1	Human monocytic leukemia cells
HMF	Hydroxymethylfurfural
HRP	Horse radish peroxidase
H_2O_2	Hydrogen peroxide
IRL	Industrial Research Limited
IU	International Unit
IL-1	Interleukin-1

IL-6	Interleukin-6
Kg	Kilogram
KGy	kilo Grays
KDa	Kilo Daltons
<	Less than
L	Litre
LPS	Lipopolysaccharide
Ltd	Limited
$LiNO_3$	Lithium nitrate
pH	-Log $[H^+]$
MS	Mass spectroscopy
MHz	Megahertz
MGO	Methylglyoxide
CH_3I	Methyl iodide
MeOH	Methanol
ML	Millilitre
µL	Microliter
mg	Milligram
µm	Micrometer
µM	Micromolar
mM	Millimolar
mm	Millimetre
min	Minutes
M	Molar
ng	Nanogram
nm	Nanomolar
N_2	Nitrogen
NMR	Nuclear magnetic resonance
ppm	Part per million
%	Percent
% (w/v)	Percent by weight per volume
PMA	Phorbol 12-myristate 13-acetate
PBS	Phosphate Buffered Saline
PBS-T	Phosphate Buffered Saline -Tween 20

PmB	Polymixin B
rpm	Revolution per minute
RPMI	Roswell park memorial institute medium
Complete RPMI	RPMI medium supplemented with 10 % FBS, penicillin at 50 IU units/mL and streptomycin at 5 µg/mL
SEC	Size-exclusion chromatography
NaN_3	Sodium azide
$NaBD_4$	Sodium borodeuteride
NaH_2PO_4	Sodium dihydrogen phosphate monohydrate
TMB	Tetra methyl benzidine
TFA	Tri fluoro acetic acid
TNF-α	Tumor necrosis factor
USP	United States Pharmacopeia
v/v	Volume per volume
WST-1 reagent	4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1, 3-benzenedisulfonate