Dimeric procyanidins as modulators of airway inflammation in the context of allergic asthma

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Declaration

It is hereby declared that this thesis has been composed by the undersigned Sara L. Coleman for the degree of Doctor of Philosophy (PhD) at Massey University. This work has not been presented in any previous application for a degree. All work was performed by the undersigned unless otherwise stated in the text. All sources of information have been specifically acknowledged in the text.

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Abstract

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Procyanidins are polyphenolic compounds that have come to be known as biologically active in the context of promoting human health. Epidemiological evidence suggests that populations that consume diets rich in procyanidins are less susceptible to inflammatory diseases. Allergic asthma is an inflammatory lung disease with an estimated 100 million affected individuals worldwide, with New Zealand having the world's second highest rate. Inflammation at the airway epithelium and infiltration of immune cells, specifically eosinophils, into the lung tissue are two central characteristics of allergic asthma. Thymic stromal lymphopoietin (TSLP) and eotaxin isoforms, eotaxin-1 (CCL11) and eotaxin-3 (CCL26), are three biomarkers of airway inflammation produced by the epithelium. Cell culture models were successfully optimized for CCL11 and CCL26 production in A549 cells. Investigation of procyanidins effect on epithelial TSLP production was not possible because TSLP production in A549 cells was undetectable. Data suggests that dimeric A-type linked procyanidin A2, but not B-type linked procyanidin B1 or B2, is capable of inhibiting IL-4-induced CCL11 production when incubated on A549 cells prior to an inflammatory insult. Co-incubation of A549 cells with procyanidin A2 and procyanidin B2 demonstrated no evidence of a synergistic relationship for inhibiting cytokine-

induced CCL11 production. Similarly, A549 cells exposed to procyanidin A2, and to a lesser extent procyanidin B2, had reduced production of cytokine-induced CCL26 production. An inhibition time course demonstrated procyanidin A2 had greatest inhibition efficacy on cytokine-induced CCL26 production when incubated for 2 h prior to an inflammatory insult. Comparison of procyanidin A2 inhibition to the known CCL26 inhibitor, IFNγ, demonstrated that procyanidin A2 and IFNγ did not share the same temporal inhibition patterns. Furthermore, experiments investigating concomitant incubation of procyanidin A2 and IFNγ demonstrated that procyanidin A2 could interfere with IFNγ–mediated CCL26 inhibition. Two possible mechanisms responsible for the procyanidin A–mediated inhibition of cytokine-induced CCL11 and CCL26 were investigated: the modulation of cytokine receptor expression, and modulation of plasma membrane fluidity. However, there was no evidence to support either of these modes of action. The data presented in this thesis collectively demonstrate the ability of procyanidin A2 to inhibit cytokine-induced eotaxin production from the lung epithelium *in vitro* and support further investigation of procyanidin A2 as a preventative approach for managing airway inflammation.

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Abbreviations

16HBE14o-	human airway epithelial cell-line
A549	human alveolar epithelial cell-line
AD	Alzheimer's disease
AP-1	activator protein-1
APC	allophycocyanin fluorophore
ARE	antioxidant response element
B2G2	procyanidin B2 3,3"-di-O-gallate
BALF	bronchoalveolar lavage fluid
BD	Becton Dickinson
BEAS-2B	human bronchial epithelial cell-line
BSA	bovine serum albumin
CA	catechin
Caco2	human colon epithelial cell-line
Calu-3	human lung epithelial cell-line
Calu-6	human lung epithelial cell-line
cAMP	cyclic adenosine monophosphate
CCL11	eotaxin-1
CCL24	eotaxin-2
CCL26	eotaxin-3
CCL3	macrophage inflammatory protein 1-alpha
CCR3	C-C chemokine receptor type 3
CD4+	cluster of differentiation 4 positive
CgC	common gamma chain
CO_2	carbon dioxide
COPD	chronic obstructive pulmonary disease
COX-2	cyclooxygenase-2
CSB	cell staining buffer
-Cy™7	cyanine 7 fluorophore
DC	dendritic cell
DiO	3,3'-dioctadecyloxacarbocyanine perchlorate
DMEM	Dulbecco's modified eagle medium
DMEM/F-12	DMEM with Nutrient Mixture F-12
DMSO	dimethyl sulfoxide
DP	degree of polymerization
DPH	1,6-diphenyl-1,3,5-hexatrience
EC	epicatechin
EDN	eosinophil-derived neurotoxin
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
em	emission

EPA	eicosapentaenoic acid
ERK1/2	extracellular-regulated kinase 1 and 2
ex	excitation
F1	first filial generation
FBS	foetal bovine serum
FceRI	high-affinity IgE receptor
FITC	fluorescein
FLVR	*Faecalibacterium*, *Lachnospira*, *Veillonella,* and *Rothia*
FSC	forward scatter
GM-CSF	granulocyte-macrophage colony-stimulating factor
H_2O_2	hydrogen peroxide
HeLa	human cervix epithelial cell-line
HepG2	human liver epithelial cell-line
HRP	horseradish peroxidase
IBD	inflammatory bowel disease
IFNγ	interferon gamma
IgE	immunoglobulin E
IgG1	immunoglobulin G1
IL	interleukin
IL-13Rα1	interleukin 13 receptor alpha one
IL-4Rα	interleukin 4 receptor alpha
ILC	innate lymphoid cell
ILC2	ILC type 2
IM9	human peripheral blood B lymphoblast cell-line
JAK	janus kinase
JNK1/2	C-Jun N-terminal kinase 1 and 2
kDa	kilo Dalton
LDH	lactate dehydrogenase
LPS	lipopolysaccharide
MALDI-TOF MS	matrix-assisted laser desorption/ionization with time-of-flight mass spectrometer
MAPK	mitogen-activated protein kinase
mRNA	messenger RNA
NAD^+	nicotinamide adenine dinucleotide (oxidized)
NADH	nicotinamide adenine dinucleotide (reduced)
NFAT	nuclear factor of activated T cells
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NO	nitric oxide
Nrf2	nuclear factor E2-related factor 2
NS	not significant
NZ	New Zealand
OVA	ovalbumin
Ox40L	Ox40 ligand

PBS	phosphate buffered saline
PD-L1	programmed death ligand 1
PE	phycoerythrin fluorophore
PerCP	peridinin chlorophyll protein fluorophore
PFR	Plant & Food Research
PMT	photomultiplier tube
Procy	procyanidin
PSN	penicillin streptomycin neomycin antibiotic mixture
RPMI	Roswell Park Memorial Institute
SCFA	short chain fatty acid
SEM	standard error mean
SPTS	Science Publication Tracking System
SSC	side scatter
STAT	signal transducers and activation of transcription
T reg	T regulatory cell
TEER	transepithelial electrical resistance
Th1	T-helper 1
Th2	T-helper 2
THLE-2	human liver epithelial cell-line
TMB	3,3'5,5'-tetramethylbenzidine
TNF R1	tumour necrosis factor receptor one
TNFα	tumour necrosis factor alpha
TSLP	thymic stromal lymphopoietin
UK	United Kingdom
US	United States
USDA	United States Department of Agriculture
UV	ultraviolet
WST-1	water soluble tetrazolium-1

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External Contributions

Portions of this thesis have been published elsewhere in the form of a book chapter, research articles, and a review article. Original drafts of these publications were solely written by Sara Coleman and then subsequently edited by co-authors of each publication. Manuscripts were submitted to Science Publication Tracking system (SPTS) at PFR, a compulsory internal process which includes scientific peer review and professional editing. All portions of this thesis not published elsewhere, are the product of Sara L Coleman alone.

A declaration of contribution detailing the specifics is included at the beginning of each chapter.

Outputs

Publications

Coleman, S.L.; Hurst, R.D.; Sawyer, G.M.; Kruger, M.C. Fruit Procyanidins: Modulating Inflammation to Promote Health. In: Sullivan I, editor. Proanthocyanidins: Food Sources, Antioxidant Properties, and Health Benefits. New York: Nova Science Publishers, Inc.; 2015. p. 73-97.

Coleman, S.L.; Hurst, R.D.; Sawyer, G.M.; Kruger, M.C.; The in vitro evaluation of isolated procyanidins as modulators of cytokine-induced eotaxin production in human alveolar epithelial cells. J. Berry Res. 2016; 5: 115-124; DOI: 10.3233/JBR-160121

Coleman, S.L.; Kruger, M.C.; Sawyer, G.M.; Hurst, R.D. Procyanidin A2 Modulates IL-4-Induced CCL26 Production in Human Alveolar Epithelial Cells. Int. J. Mol. Sci. 2016, 17, 1888; DOI: 10.3390/ijms17111888

Coleman, S.L.; Shaw, O.M. Progress in our understanding of allergic asthma pathology supports the potential of fruit proanthocyanidins as modulators of airway inflammation. 2017 (Under Review)

Abstracts for Publications can be seen in Appendix I

Presentations

Presentations:

Dec 2014	Confirmation Presentation at Massey University, Palmerston North. New Zealand
July 2015	Oral Presentation at NZASI Conference Auckland, New Zealand
October 2015	Poster Presentation at Berry Health Benefits Symposium Conference, Madison, Wisconsin, USA
June 2016	Oral Presentation at Joint Graduate School of Horticulture and Food Enterprise, Massey University, Palmerston North, New Zealand

Additional Scientific Education:

July 2014	Attendance NZASI Conference Palmerston North, NZ
October 2015	US:NZ Science Workshop- Building the Health Claims Dossier for Berry Fruits
April 2016	Polychromatic Flow Cytometry Roadshow, Wellington, NZ