Role of antioxidants in rhinovirus-infected airway epithelial cells

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MSc

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Statement of originality

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List of abbreviations

1α,25(OH)$_2$D$_3$: 1α-25-dihydroxycholecalciferol or 1α-25-dihydroxyvitaminD$_3$
2A^{pro}: viral protease 2A
3CD^{pro}: viral protease CD
3C^{pro}: viral protease 3C
A549: human alveolar epithelial cells line
AD: adenovirus
AP-1: activator protein-1
Br$^-$: bromide
BECs: bronchial/airway epithelial cells
Calu-3: human airway epithelial cells line
CARDS: caspase activation and recruiting domain
CAT: catalase
Cl$^-$: cloride
COPD: Chronic obstructive pulmanory disease
COX-2: cyclooxygenase-2
CuZnSOD: copper-zinc superoxide dismutase
CYP: cytochrome P$_{450}$
DMSO: dimethyl sulfoxide
dsRNA: double-stranded ribonucleic acid
ELISA: enzyme-link immunosorbent assay
ELAM-1: endothelial leukocyte adhesion molecule-1
EPO: eosinophil peroxidase
FCS/DMEM: foetal calf serum/Dulbecco's modified eagle medium
FCS/MEM: foetal calf serum/minimum essential medium
FEV: Forced expiratory volume in 1 second
FVC: Forced vital capacity
GAPDH: glyceraldehyde-3-phosphate dehydrogenase
γ-GT: gamma-glutamyl transpeptidase
GM-CSF: granulocyte-monocyte-colony stimulating factor
GSH: reduced glutathione
GSHPx: glutathione peroxidase
GSSG: oxidized glutathione
H_2O_2: hydrogen peroxide
Hep-2: human respiratory (larynx) epithelial cells line
HOBr: hypobromous acid
HOCl: hypochlorous acid
ICAM-1: intercellular adhesion molecule-1
IFN: interferon
IFN-k: interferon-kappa
IFN-β: interferon-beta
IFN-γ: interferon-gamma
IFN-λ1: interferon-lambda 1 (interleukin-28A)
IFN-λ2: interferon-lambda 2 (interleukin-28B)
IFN- λ3: interferon-lambda 3 (interleukin-29)
IFN-ω: interferon omega
IKK: IK-B kinase complex
IL-13: interleukin-13
IL-1β: inteleukin-1 beta

IL-4: interleukin-4
IL-5: interleukin-5
IL-6: interleukin-6
IL-8: interleukin-8
IL-9: interleukin-9
iNOS: inducible nitric oxide synthase
IP-10: interferon-gamma-induced protein-10
IRES: internal ribosome entry site
IRF-3: interferon regulatory factor-3
IRF-7: interferon regulatory factor-7
LPS: lipopolysaccharide
MAVS: mitochondrial antiviral signaling protein
MDA-5: melanoma differentiation associated gene-5
MDCK: madin-darby canine kidney epithelial cells line
m-ICAM-1: membranous-intercellular adhesion molecule-1
MMP-9: matrix metalloproteinase-9
MnSOD: manganese superoxide dismutase
MPO: myloperoxidase
MRC-5: human lung fibrolast cells line
mRNA: messenger ribonucleic acid
NAD^+: nicotinamide adenine dinucleotide
NADPH: reduced form of nicotinamide adenine dinucleotide phosphate
NF-κB: nuclear factor-kappa B
O_2^-: superoxide radical
P1: viral genome encoded structural protein 1
P2: viral genome encode non-structural protein 2
P3: viral genome encode non-structural protein 3
PH: plecstrin homology
PI3-k: phosphotidyl inositol-3-kinase
PRR: pathogen recognition receptor
PtdIns(3)P: phosphotidyl inositol-3-phosphate
PtdIns(3,4,5)P_3: phosphotidyl inositol (3,4,5) triphosphate
PtdIns(4,5)P_2: phosphotidyl inositol (4,5) biphosphate
RANTES: regulated upon activation normal T-cell expressed and secreted
RD-ICAM-1: rhabdomyosarcoma expressing intercellular adhesion molecule-1
RIG-I: retinoic acid inducible gene
RNA: ribonucleic acid
ROS: reactive oxygen species
RSV: respiratory syntical virus
RT-PCR: real time-polymerase chain reaction
RV: Human rhinovirus
RV43: human rhinovirus-43 (rhinovirus species A and major group)
RV1B: human rhinovirus-1B (rhinovirus species A and minor group)
RV-A: human rhinovirus species A
RV-B: human rhinovirus species B
RV-C: human rhinovirus species C
RV-D: human rhinovirus species D
SEM: standard error of mean
ssRNA: single-stranded ribonucleic acid
TBARS: thiobarbituric reactive substances

Th: T helper cells (subgroup of lymphocytes)
THF: tetrahydrofuoran
TICAM: Toll-interleukin 1 receptor domain (TIR)-containing adaptor molecule-1
TLR: toll like receptor
TNF-α: tumor necrosis factor-alpha
TRIF: TIR domain-containing adapter inducing interferon-beta
URTI: upper respiratory tract infection
UTR: untranslated region
VCAM-1: intervascular adhesion molecule-1
VP1: viral capsid protein 1
VP2: viral capsid protein 2
VP3: viral capsid protein 3
VP4: viral capsid protein 4
VPg: viral small protein
vRNA: viral ribonucleic acid
XO: xanthine oxidase
Zn: Ion zinc
$ZnSO_4$: Zinc sulphate

Publication arising from this thesis

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2. FH Addnan, PAB Wark, LG Wood, ML Garg: Antiviral activity of antioxidants against rhinovirus infection. Conference Abstracts Nutrition Society of Australia and Nutrition Society of New Zealand 2011, Australasian Medical Journal 2011, 4, 12, 739-788.

3. FH Addnan, LG Wood, ML Garg, PAB Wark: Antiviral effects of antioxidants on human rhinovirus. Special Issue: Abstracts of the Thoracic Society of Australia & New Zealand and the Australian & New Zealand Society of Respiratory Science 2012 Annual Scientific Meetings, 30 March-4 April 2012, National Convention Centre, Canberra, ACT. Respirology 2012, 17 (Suppl. 1), 12-41.

4. Faizul H. Addnan, Peter A.B. Wark, Lisa G. Wood, Manohar L. Garg: Effects of antioxidants on rhinovirus replication and rhinovirus-induced inflammation in cultured airway epithelial cells. *J Nutr Biochem* (Manuscript in preparation)

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Abstract

Human rhinovirus are associated with the majority of exacerbations of asthma and chronic obstructive pulmonary disease. The epithelial cells of the airway are the primary target for invading rhinovirus and the alterations on the airway epithelium by the virus are believed to be central in enhancing the airway inflammation that leads to asthma exacerbations. The development of a conventional vaccine is not practical to fight against rhinovirus, due to the fact that there are more than 100 serotypes. Natural agents capable of interfering with viral replication warrant exploration, because as yet, no licensed effective antiviral is currently available. Hence, this thesis is conducted to provide a promising candidate against rhinovirus infection.

We utilized natural potent antioxidant compounds including resveratrol, lycopene, zinc and vitamin D at physiologically relevant concentrations, to prevent inflammatory response of airway epithelial cells induced by rhinovirus. In this thesis, we studied the anti-inflammatory effect of resveratrol, lycopene, zinc and vitamin D against the major group of human rhinovirus, (consist of 90% rhinovirus serotypes) which is using intercellular adhesion molecule-1 on host cells to gain infection. We found that enriched Calu-3 cells with those antioxidant compounds prior to rhinovirus infection, significantly prevent the virus from replicating efficiently. However, the antioxidants failed to significantly decrease the inflammatory response of Calu-3 cells induced by rhinovirus. Rhinovirus infection cause significant secretion of interleukin-6, interleukin-8 and interferon-gamma-induced protein-10 into the cultured media, hence confirming the model used for investigating the effect of antioxidant compounds against the virus.

Thorough mechanism studies to unfold antioxidants' mode of action against rhinovirus replication were conducted. The study revealed that phosphotidyl inositol-3-kinase is required during RV internalisation, and enriched Calu-3 cells with resveratrol, lycopene and vitamin D decreased the activation level of phosphotidyl inositol-3-kinase, hence explained the significant decrease of viral titers observed earlier in the rhinovirus infection study. Verification studies were done using wortmannin which is a specific inhibitor of phosphotidyl inositol-3-kinase and visualizing AlexaFluor 555-labelled rhinovirus entry into Calu-3 cells by confocal microscopy. Antioxidant compounds were found not to have any significant effect in the course of viral translation and viral replication steps.

Resveratrol, lycopene, vitamin D and zinc, were demonstrated to have beneficial roles in limiting rhinovirus replication in Calu-3 cells. Preventing or ameliorating rhinovirus replication will hopefully bring significant impact towards managing asthmatic patients who are at high risk of suffering rhinovirus-induced asthma exacerbations.