Role of antioxidants in rhinovirus-infected airway epithelial cells

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MSc

A thesis submitted for the degree of Doctor of Philosophy

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Statement of originality

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# Table of content

Role of antioxidants in rhinovirus-infected airway epithelial cells
Statement of originality.............................................................. II
Acknowledgement........................................................................... III
Table of content............................................................................ IV
List of figures.................................................................................. VII
List of tables................................................................................... IX
List of abbreviations....................................................................... X
Publication arising from this thesis.................................................. XIII
Abstract......................................................................................... XIV

## Chapter 1: Introduction

1.1 Introduction............................................................................... 2
1.2 Etiological factors in the development of asthma......................... 5
1.3 Diet and asthma.......................................................................... 8
1.4 Inflammation in asthma............................................................. 9
  1.4.1 Mediators of inflammation in asthma............................... 10
  1.4.2 Cytokines.......................................................................... 11
  1.4.3 Pro-inflammatory cytokines............................................ 11
  1.4.4 Transcription factors...................................................... 12
1.5 Oxidative stress in asthma........................................................ 14
1.6 Viruses and asthma.................................................................... 15
  1.6.1 Human rhinoviruses....................................................... 16
1.7 Rhinovirus, oxidative stress and cellular dysfunction................... 18
1.8 Rhinovirus and innate immunity............................................... 20
1.9 Replication of rhinoviruses........................................................ 22
  1.9.1 Rhinoviruses and PI3-kinase........................................... 26
1.10 Antioxidants and PI3-kinase..................................................... 28
1.11 Antioxidants and respiratory diseases........................................ 29
  1.11.1 Resveratrol..................................................................... 29
    1.11.1.1 Description, sources and measurement...................... 29
    1.11.1.2 Resveratrol and respiratory diseases..................... 31
    1.11.1.3 Antioxidant effects-\textit{in vitro} and \textit{in vivo} evidence... 32
    1.11.1.4 Anti-inflammatory effects-\textit{in vitro} and \textit{in vivo}
                 evidence............................................................. 34
  1.11.2 Lycopene......................................................................... 36
    1.11.2.1 Description, sources and measurement...................... 36
    1.11.2.2 Lycopene and respiratory diseases........................ 37
    1.11.2.3 Antioxidant effects-\textit{in vitro} and \textit{in vivo} evidence... 38
    1.11.2.4 Anti-inflammatory effects-\textit{in vitro} and \textit{in vivo}
                 evidence............................................................. 40
  1.11.3 Zinc............................................................................... 41
    1.11.3.1 Description, sources and measurement...................... 41
    1.11.3.2 Zinc and respiratory diseases................................ 42
    1.11.3.3 Antioxidant effects-\textit{in vitro} and \textit{in vivo} evidence... 43
    1.11.3.4 Anti-inflammatory effects-\textit{in vitro} and \textit{in vivo}
                 evidence............................................................. 44
  1.11.4 Vitamin D...................................................................... 45

IV
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.11.4.1</td>
<td>Description, sources and measurement</td>
<td>45</td>
</tr>
<tr>
<td>1.11.4.2</td>
<td>Vitamin D and respiratory diseases</td>
<td>47</td>
</tr>
<tr>
<td>1.11.4.3</td>
<td>Antioxidant effects <em>in vitro</em> and <em>in vivo</em> evidence</td>
<td>48</td>
</tr>
<tr>
<td>1.11.4.4</td>
<td>Anti-inflammatory effects <em>in vitro</em> and <em>in vivo</em> evidence</td>
<td>49</td>
</tr>
<tr>
<td>1.12</td>
<td>Conclusion</td>
<td>50</td>
</tr>
<tr>
<td>1.13</td>
<td>Hypothesis</td>
<td>51</td>
</tr>
<tr>
<td>2.1</td>
<td>Introduction</td>
<td>54</td>
</tr>
<tr>
<td>2.2</td>
<td>Cell cultures experiments</td>
<td>55</td>
</tr>
<tr>
<td>2.2.1</td>
<td><em>In vitro</em> epithelial cell culture</td>
<td>55</td>
</tr>
<tr>
<td>2.2.2</td>
<td>Preparing virus stock and TCID50 assay</td>
<td>55</td>
</tr>
<tr>
<td>2.2.3</td>
<td>Rhinovirus-43 fluorescent labelling</td>
<td>57</td>
</tr>
<tr>
<td>2.2.3.1</td>
<td>Virus purification</td>
<td>57</td>
</tr>
<tr>
<td>2.2.3.2</td>
<td>Fluorescent labelling of virus</td>
<td>58</td>
</tr>
<tr>
<td>2.2.4</td>
<td>Confocal microscopy</td>
<td>58</td>
</tr>
<tr>
<td>2.2.4.1</td>
<td>Collagen-coated coverslip</td>
<td>58</td>
</tr>
<tr>
<td>2.2.4.2</td>
<td>Cells preparation for confocal microscopy</td>
<td>59</td>
</tr>
<tr>
<td>2.2.5</td>
<td>Preparing medium containing resveratrol-DMSO</td>
<td>59</td>
</tr>
<tr>
<td>2.2.6</td>
<td>Preparing medium containing lycopene</td>
<td>60</td>
</tr>
<tr>
<td>2.2.7</td>
<td>Preparing medium containing zinc salt</td>
<td>60</td>
</tr>
<tr>
<td>2.2.8</td>
<td>Preparing medium containing 1α,25-dihydroxyvitaminD3</td>
<td>61</td>
</tr>
<tr>
<td>2.2.9</td>
<td>Supplementation of Calu-3 with resveratrol, lycopene, zinc, vitamin D3</td>
<td>61</td>
</tr>
<tr>
<td>2.2.10</td>
<td>Primers</td>
<td>61</td>
</tr>
<tr>
<td>2.2.11</td>
<td>Common buffers for protein analysis</td>
<td>62</td>
</tr>
<tr>
<td>2.3</td>
<td>Biochemical analysis</td>
<td>63</td>
</tr>
<tr>
<td>2.3.1</td>
<td>Resveratrol analysis</td>
<td>63</td>
</tr>
<tr>
<td>2.3.1.1</td>
<td>Resveratrol extraction</td>
<td>63</td>
</tr>
<tr>
<td>2.3.1.2</td>
<td>HPLC-Resveratrol</td>
<td>64</td>
</tr>
<tr>
<td>2.3.2</td>
<td>Analysis of inflammation biomarkers</td>
<td>64</td>
</tr>
<tr>
<td>2.3.2.1</td>
<td>Enzyme-Linked Immunosorbent Assay (ELISA)</td>
<td>65</td>
</tr>
<tr>
<td>2.3.3</td>
<td>Analysis of cellular apoptosis, necrosis and viability</td>
<td>66</td>
</tr>
<tr>
<td>2.3.4</td>
<td>Analysis of intracellular adhesion molecule-1 (ICAM-1) surface receptor expression</td>
<td>67</td>
</tr>
<tr>
<td>2.3.5</td>
<td>Total protein concentration quantification</td>
<td>67</td>
</tr>
<tr>
<td>2.3.6</td>
<td>SDS-PAGE electrophoresis and western blotting</td>
<td>68</td>
</tr>
<tr>
<td>2.3.7</td>
<td>RNA analysis</td>
<td>69</td>
</tr>
<tr>
<td>2.3.7.1</td>
<td>RNA harvesting and extraction</td>
<td>69</td>
</tr>
<tr>
<td>2.3.7.2</td>
<td>Reverse transcription of RNA to cDNA</td>
<td>70</td>
</tr>
<tr>
<td>2.3.7.3</td>
<td>Real-time quantitative polymerase chain reaction (RT-qPCR)</td>
<td>70</td>
</tr>
<tr>
<td>2.3.7.4</td>
<td>Viral RNA RT-qPCR</td>
<td>71</td>
</tr>
<tr>
<td>2.3.7.5</td>
<td>RNA interference</td>
<td>73</td>
</tr>
</tbody>
</table>

**Chapter 2: General Materials and Methods**  | 53 |

**Chapter 3: Resveratrol supplementation of cultured airway epithelial cells**  | 74 |

**Chapter 4: Effects of dietary antioxidants on rhinovirus replication and rhinovirus induced inflammation in cultured airway epithelial cells**  | 90 |
<table>
<thead>
<tr>
<th>Chapter 5: Mechanism by which antioxidants reduce viral replication in rhinovirus-infected cultured airway epithelial cells</th>
<th>122</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 6: Effects of antioxidant treatment on rhinovirus internalization in infected cultured airway epithelial cells and the role of PI3-kinase</td>
<td>148</td>
</tr>
<tr>
<td>Chapter 7: General discussions and conclusions</td>
<td>166</td>
</tr>
<tr>
<td>References</td>
<td>174</td>
</tr>
</tbody>
</table>
# List of figures

<table>
<thead>
<tr>
<th>Figure ID</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.6.1.1</td>
<td>Enterovirus genome organization</td>
<td>18</td>
</tr>
<tr>
<td>Figure 1.9.1</td>
<td>Overview of rhinovirus (major group) replication</td>
<td>24</td>
</tr>
<tr>
<td>Figure 1.9.2</td>
<td>General overview on rhinovirus endocytosis pathway</td>
<td>26</td>
</tr>
<tr>
<td>Figure 1.11.1.1.1</td>
<td>Chemical structure of trans-resveratrol</td>
<td>30</td>
</tr>
<tr>
<td>Figure 1.11.1.1.2</td>
<td>Related structures of resveratrol molecule (top 3) and metabolites of resveratrol (bottom 3)</td>
<td>31</td>
</tr>
<tr>
<td>Figure 1.11.2.1.1</td>
<td>Molecular structure of lycopene</td>
<td>37</td>
</tr>
<tr>
<td>Figure 1.11.4.1.1</td>
<td>The conversion of provitamin D$_3$ to vitamin D$_3$</td>
<td>47</td>
</tr>
<tr>
<td>Figure 1.13.1</td>
<td>Hypothesised mechanisms of antioxidant (for example RESV) action on the pro-inflammatory response to viruses</td>
<td>52</td>
</tr>
<tr>
<td>Figure 3.3.1</td>
<td>Calu-3 cells 100% confluent in the bottom of 24-well plate</td>
<td>78</td>
</tr>
<tr>
<td>Figure 3.3.2</td>
<td>Calu-3 cells after incubation with resveratrol for 24 hours</td>
<td>79</td>
</tr>
<tr>
<td>Figure 3.3.3</td>
<td>Calu-3 cells after incubation with 1µM of vitamin D for 24 hours</td>
<td>79</td>
</tr>
<tr>
<td>Figure 3.3.4</td>
<td>Calu-3 cells after incubation with 2.5µg/ml of lycopene for 24 hours</td>
<td>79</td>
</tr>
<tr>
<td>Figure 3.3.5</td>
<td>Calu-3 cells after incubation with 25µM of zinc for 24 hours</td>
<td>80</td>
</tr>
<tr>
<td>Figure 3.3.6</td>
<td>Cytotoxicity of resveratrol on Calu-3 cells</td>
<td>81</td>
</tr>
<tr>
<td>Figure 3.3.7</td>
<td>Concentration of resveratrol (aglycone) in extracellular (media) Calu-3 cells</td>
<td>82</td>
</tr>
<tr>
<td>Figure 3.3.8</td>
<td>Resveratrol metabolites in extracellular (media) Calu-3 cells</td>
<td>82</td>
</tr>
<tr>
<td>Figure 3.3.9</td>
<td>Resveratrol aglycone (within 6 hours) in extracellular (media) Calu-3 cells</td>
<td>84</td>
</tr>
<tr>
<td>Figure 3.3.10</td>
<td>Resveratrol metabolites (within 6 hours) in extracellular (media) calu-3 cells</td>
<td>84</td>
</tr>
<tr>
<td>Figure 3.3.11</td>
<td>Resveratrol metabolites (within 6 hours) intracellular of Calu-3 cells after 50 and 100µM of resveratrol administration</td>
<td>85</td>
</tr>
<tr>
<td>Figure 3.3.12</td>
<td>Resveratrol metabolites (within 6 hours) intracellular of Calu-3 cells after 5, 10 and 25µM of resveratrol administration</td>
<td>85</td>
</tr>
<tr>
<td>Figure 4.1.1</td>
<td>Airway epithelial cells is the primary target of rhinovirus and is implicated in the initiation of innate and subsequent activation of adaptive immunity</td>
<td>93</td>
</tr>
<tr>
<td>Figure 4.3.1</td>
<td>Effect of zinc at various concentrations on (a) rhinovirus-1B (RV1B) and (b) rhinovirus-43 (RV43) replication in Calu-3 cells</td>
<td>104</td>
</tr>
<tr>
<td>Figure 4.3.2</td>
<td>Effect of vitamin D at various concentrations on (a) rhinovirus-1B (RV1B) and (b) rhinovirus-43 (RV43) replication in Calu-3 cells</td>
<td>105</td>
</tr>
<tr>
<td>Figure 4.3.3</td>
<td>Effect of lycopene at various concentrations on (a) rhinovirus-1B (RV1B) and (b) rhinovirus-43 (RV43) replication in Calu-3 cells</td>
<td>106</td>
</tr>
<tr>
<td>Figure 4.3.4</td>
<td>Effect of resveratrol at various concentrations on (a) rhinovirus-1B (RV1B) and (b) rhinovirus-43 (RV43) replication in Calu-3 cells</td>
<td>107</td>
</tr>
<tr>
<td>Figure 4.3.5</td>
<td>Combined experiments on effect of antioxidant on (a) rhinovirus-1B (RV1B) and (b) rhinovirus-43 (RV43) replication in Calu-3 cells</td>
<td>108</td>
</tr>
<tr>
<td>Figure 4.3.6</td>
<td>Effects of antioxidants on rhinovirus-43 (RV43)-induced pro-inflammatory cytokine, IL-8</td>
<td>110</td>
</tr>
</tbody>
</table>
Figure 4.3.7. Effects of antioxidants on rhinovirus 43 (RV43)-induced pro-inflammatory cytokine, IL-6

Figure 4.3.8. Effects of antioxidants on rhinovirus 43 (RV43)-induced pro-inflammatory cytokine, IP-10

Figure 4.3.9. Effects of antioxidants on viability of Calu-3 cells infected with rhinovirus 43 (RV43)

Figure 4.3.10. Effects of antioxidants on (a) apoptotic and (b) necrotic/late apoptotic of Calu-3 cells infected with rhinovirus 43 (RV43)

Figure 5.3.1. Membranous ICAM-1 protein expression at (a) 6 hours, (b) 48 hours of Calu-3 cells supplemented with antioxidants infected with RV43

Figure 5.3.2. The activation of PI3-kinase on RV43 infected Calu-3 cells is inhibited by resveratrol, vitamin D and lycopene

Figure 5.3.3. Inhibition of PI3-kinase by PI3-kinase inhibitor, wortmannin on RV43 replication in Calu-3 cells

Figure 5.3.4. Wortmannin reduces RV43 replication in Calu-3 cells

Figure 5.3.5. Effects of wortmannin (Wort) on viability of Calu-3 cells infected with RV43

Figure 5.3.6. Effect of zinc and resveratrol on RV43 replication

Figure 5.3.7. Effect of vitamin D and lycopene on RV43 replication

Figure 5.3.8. Effect of antioxidant-enriched cells on RV43 mediated cellular cleavage of eIF4GI at 6 hours after infection

Figure 5.3.9. Effect of antioxidant-enriched cells on RV43 mediated cellular cleavage of eIF4GI at 24, 48 and 72 hours after infection

Figure 5.3.10. RV43 entry inhibition by resveratrol, vitamin D and lycopene

Figure 6.1.1. Hypothesis on the direct effect of antioxidant on PI3-kinase during viral internalization via silencing regulatory protein of PI3-kinase, PTEN

Figure 6.2.1. Schematic diagram of the study design to assess role of antioxidants in inhibiting activation of PI3-kinase via silencing PTEN on RV replication

Figure 6.3.1. The used of siRNA at concentrations below or at 40nM does not cause cells toxicity

Figure 6.3.2. Percentage (%) of mRNA GAPDH knocked down when siRNA to GAPDH was administered to Calu-3 cells at 48 hours of incubation times

Figure 6.3.3. Percentage (%) of PTEN mRNA knocked down in Calu-3 cells

Figure 6.3.4. PTEN mRNA gene expression in RV43 infected-antioxidant enriched-Calu-3 cells

Figure 6.3.5. PTEN mRNA knocked down decreases the rate of viral titer in Calu-3 cells

Figure 6.3.6. siRNA against PTEN markedly affects Calu-3 cells viability
List of tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.3.1</td>
<td>The relationship between antioxidant nutrients (including lycopene) and asthma</td>
<td>9</td>
</tr>
<tr>
<td>2.2.10.1</td>
<td>Commercial assays used and list of primer-probes for RT-qPCR</td>
<td>62</td>
</tr>
<tr>
<td>2.2.10.2</td>
<td>List of primer-probes and sequences for RV in RT-qPCR</td>
<td>62</td>
</tr>
<tr>
<td>2.3.6.1</td>
<td>List of antibodies and dilution used to determine protein of interest</td>
<td>69</td>
</tr>
<tr>
<td>2.3.7.4.1</td>
<td>Preparation of 20X RV mix</td>
<td>72</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Physiological concentration of antioxidants in human: resveratrol, zinc, lycopene and vitamin D</td>
<td>87</td>
</tr>
</tbody>
</table>
List of abbreviations

$1\alpha,25(OH)_{2}D_{3}$: 1$\alpha$-25-dihydroxycholecalciferol or 1$\alpha$-25-dihydroxyvitaminD$_{3}$
2A$^{\text{pro}}$: viral protease 2A
3CD$^{\text{pro}}$: viral protease CD
3C$^{\text{pro}}$: viral protease 3C
A549: human alveolar epithelial cells line
AD: adenovirus
AP-1: activator protein-1
Br$: bromide
BECs: bronchial/airway epithelial cells
Calu-3: human airway epithelial cells line
CARDS: caspase activation and recruiting domain
CAT: catalase
Cl$: chloride
COPD: Chronic obstructive pulmonary 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$_{2}$O$_{2}$: hydrogen peroxide
Hep-2: human respiratory (larynx) epithelial cells line
HOB$: hypobromous acid
HOCl$: 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: interleukin-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: myeloperoxidase
MRC-5: human lung fibroblast cells line
mRNA: messenger ribonucleic acid
NAD+: nicotinamide adenine dinucleotide
NADPH: reduced form of nicotinamide adenine dinucleotide phosphate
NF-κB: nuclear factor-kappa B
O2·−: 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
P13-k: phosphotidyl inositol-3-kinase
PRR: pathogen recognition receptor
PtdIns(3)P: phosphotidyl inositol-3-phosphate
PtdIns(3,4,5)P3: phosphotidyl inositol (3,4,5) triphosphate
PtdIns(4,5)P2: 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
ZnSO₄: Zinc sulphate
Publication arising from this thesis


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.