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Lycopene enrichment of cultured airway epithelial cells decreases the inflammation induced by rhinovirus infection and lipopolysaccharide

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Abstract

Rhinovirus infection results in increased release of inflammatory mediators from airway epithelial cells in asthma. As an antioxidant, lycopene offers protection from adverse effects of inflammation. The aim of this study was to find an appropriate method of lycopene enrichment of airway epithelial cells and to determine the effects of lycopene enrichment on the inflammatory response of cells infected by rhinovirus or exposed to lipopolysaccharide. Lycopene enrichment of airway epithelial cells using solubilisation in tetrahydrofuran versus incorporation in liposomes was compared. After determining that solubilisation of lycopene in tetrahydrofuran was the most suitable method of lycopene supplementation, airway epithelial cells (Calu-3) were incubated with lycopene (dissolved in tetrahydrofuran) for 24 h, followed by rhinovirus infection or lipopolysaccharide exposure for 48 h. The release of interleukin-6, interleukin-8 and interferon-gamma induced protein-10 (IP-10) and their messenger RNA levels were measured using enzyme linked immunosorbent assay and reverse transcription polymerase chain reaction, respectively. Viral replication was measured by tissue culture infective dose of 50% assay. Lycopene concentration of cells and media were analysed using high-performance liquid chromatography. Preincubation of airway epithelial cells with lycopene (dissolved in tetrahydrofuran) delivered lycopene into the cells and resulted in a 24% reduction in interleukin-6 after rhinovirus-1B infection, 31% reduction in IP-10 after rhinovirus-43 infection and 85% reduction in rhinovirus-1B replication. Lycopene also decreased the release of IL-6 and IP-10 following exposure to lipopolysaccharide. We conclude that lycopene has a potential role in suppressing rhinovirus induced airway inflammation.

Keywords: Lycopene; Antioxidant; Inflammation; Rhinovirus; Acute asthma

1. Introduction

Rhinovirus (RV) is the most common cause of the common cold [1] and the major cause of exacerbation in asthma among adults [2] and children [2,3]. Rhinovirus infection can also worsen airway obstruction in asthmatics [4], though this mechanism is incompletely understood [5]. RVs target epithelial cells, in which they replicate [6] and initiate innate immune responses [7,8]. As a result, epithelial cells produce various inflammatory mediators that contribute to the host defense and result in increased airway inflammation [5]. This includes, interleukin-6 (IL-6) [6,9,10], interleukin-8 (IL-8) [6,10–14] and interferon-gamma induced protein-10 (IP-10) [5]. Rhinovirus infection also increases oxidative stress in airway epithelial cells with an increase in production of reactive oxygen species (ROS) [15,16]. This directly leads to the translocation of nuclear factor-κB (NF-κB) [16], which mediates the transcription of IL-6 and IL-8 [9,14,17]. During acute clinical infections, RV leads to increased permeability and the recruitment of neutrophils to the airways [18], with increased levels of IL-8 and neutrophils closely correlated to symptoms [19,20]. In asthma, we have previously shown that acute virus exacerbations are also associated with increased

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levels of sputum IL-8, neutrophils and neutrophil degranulation, and the degree of neutrophil mediated inflammation is associated with more severe symptoms and greater impairment of lung function [20].

Carotenoids are natural fat-soluble compounds [21], which act as antioxidants [22–26]. Among them, lycopene, which can be found in high concentrations in tomatoes [27], has the most potent antioxidant potential [28–30]. Lycopene has been shown to decrease inflammation in vivo [31–33] and in vitro [33,34]. Of particular interest is the ability of lycopene to reduce airway inflammation, including both neutrophil influx and activation [35]. There is a limited evidence regarding the effect of lycopene on viral infections; however, in vivo studies have reported that lycopene decreases human papillomavirus persistent risk and also decreases its clearance time [36,37]. There is no evidence regarding the effect of lycopene on the inflammatory response of airway epithelial cells when exposed to common triggers, such as rhinovirus infection and lipopolysaccharide (LPS) exposure.

The poor water solubility of lycopene has affected the ability to study its in vitro effects on cultured cells. Tetrahydrofuran (THF) has been used as a lycopene cosolvent [38,39]. Liposomes, which are spherical particles containing hydrophilic compounds in the surface and hydrophobic compounds in the core, have also been used as lycopene carriers [40,41]. THF can dissolve considerable amounts of lycopene (more than 4 mg/ml lycopene) [40]. A previous study also successfully reported using liposomes for lycopene enrichment of human umbilical vein endothelial cells and had a greater carrying capacity with a longer half life compared to THF [40,42]. However, other researchers have criticized the use of liposomes for lycopene enrichment due to carotenoid instability [43,44].

Our aim was to determine an effective means of delivering and enriching epithelial cells with lycopene. We then hypothesised that lycopene supplementation would decrease inflammation in airway epithelial cells following infection by rhinovirus.

2. Materials and methods

2.1. Airway epithelial cell culture

Airway epithelial cells (Calu-3 cells from ATCC, Manassas, VA, USA) were cultured in minimum essential medium containing 10% foetal calf serum (FCS/MEM), containing 2% penicillin-streptomycin, 1% sodium pyruvate, 1% nonessential amino acids, 1% L-glutamine and 2.2 g/L $NaHCO_3$ (all from Invitrogen, Carlsbad, CA, USA) in plastic culture plates at 37°C in the presence of 5% CO_2. All experiments were carried out in cells with >80% confluence.

2.2. Lycopene enrichment of cultured epithelial airway cells, THF versus liposomes

Two methods for delivery of lycopene were compared. Confluent Calu-3 cells (Passage 28–30) were incubated (for 24 h) with lycopene (0, 2.5, 5, 10 and 25 μg/ml) dissolved in THF (0%, 0.5%, 1%, 2% and 5% THF in cell culture medium). This was compared to lycopene (0, 5 and 10 μg/ml) carried by liposomes. The final concentration of each component in the liposome was: 100 μM monoolein, 33.3 μM oleic acid, 2 μM sodium taurocholate and 16.7 μM L-α-phosphatidylcholine, after removing THF under nitrogen steam, (Organomation Associates, Berlin, MA, USA) and sonication in 37°C water bath (Sanophon, Ultrasonic Industries, Dayton, OH, USA). After 24 h, cells were visually inspected using a microscope and both the media, and the cells were collected.

2.3. Lycopene analysis

In order to measure the extracellular and intracellular concentration of lycopene, Barua's method for high-performance liquid chromatography (HPLC) was employed [45]. All of the extraction processes were undertaken in a darkened laboratory under red light. Extracellular (500 μl of media) and intracellular (cell pellets of cell count of 20 million) lycopene were extracted from the samples by adding (a) ethanol:ethyl acetate (1:1) containing canthaxanthin as an internal standard, (b) ethyl acetate (2 times 1 ml) and (c) hexane (1 ml). After each step, samples were vortexed and centrifuged (3000×*g*, 4°C, for 5 min), and supernatants were transferred to a 10-ml tube placed in ice. Next 1-ml ultra pure water was added to the combination of supernatants in ice, vortexed and centrifuged. The supernatants were then collected in glass high-recovery 10-ml tubes and placed in a nitrogen evaporator (Organomation Associates) to evaporate ethyl acetate and hexane. Dry lycopene samples were resuspended in 100 μl of injection solvent (dichloromethane: methanol; 1:2 v/v). Samples were analysed by HPLC 1100 series (Agilent Technologies, Santa Clara, CA, USA) by injecting (5 μl, flow rate: 0.3 ml/min) into Hypersil ODS C18 column (Thermo Electron, Bellefonte, PA, USA) and detecting by the diode array detector (Agilent Technologies) at a preset 450-nm wavelength.

2.4. Rhinovirus and LPS exposure of calu-3 cells

After removing the supplementation media (as mentioned above) from Calu-3 cells (Passage 31–33), they were either infected with (a) rhinovirus-43 (RV-43) [multiplicity of infection: (MOI)=0.32] (b), rhinovirus-1B (RV-1B) (MOI=0.56) (viruses were laboratory strain provided by Woolcock Research Institute, Sydney, Australia), (c) lipopolycaccharide 1 μg/ml (from *Escherichia coli* strain 026: B6, Sigma, St Louis, MO, USA) and also (d) ultraviolet inactivated RVs. Plates were incubated for 48 h at 37°C in the presence of 5% CO_2.

2.5. Cytokine analysis

IL-6, IL-8 and IP-10 concentrations of media were measured by ELISA (R&D Systems Minneapolis, MN, USA) according to manufacturer's instructions.

2.6. Messenger RNA analysis

In order to measure mRNA levels of cytokines, the method of Grissell et al was employed, as previously described [46]. In this method the reverse transcriptase–polymerase chain reaction (RT-PCR) was used. RNA was extracted (RNeasy kit, Qiagen, Carlsbad, CA, USA), and reverse transcripted to cDNA (Qiagen) to be measured by RT-PCR according to the manufacturer's instructions.

2.7. Viral titration assay

Tissue culture infective dose of 50% ($TCID_{50}$) experiments were performed using confluent RD-ICAM-1 cells seeded in 96-well tissue culture plates (NUNC, Roskilde, Denmark). Cells were infected by either media alone or virus-containing media at varying dilutions. Serial 10-fold dilutions of the samples were prepared, and four individual wells were infected with each dilution. For titration of samples 6 dilutions were prepared. Additionally, for every dilution, two control wells were prepared with media alone. After 4 days, the plates were read, and $TCID_{50}$ was calculated. Infected wells were scored based on the cytopathic effect (CPE) seen; >50% CPE demonstrated by light microscopy was considered a positive result. Viral titers of the samples were determined by cell titration assay using RD-ICAM-1 cells, and the viral titer was calculated and expressed as tissue culture infectious dose at 50% in log value ($TCID_{50}$ $\log_{10}$) [47] and using the Karber formula for the tissue culture infective dose 50% ($TCID_{50}$) [48].

2.8. Lactate dehydrogenase assay

Lactate dehydrogenase (LDH) concentration in the media was measured by the enzymatic method on the Dade Behring RXL Dimension platform (Dade Behring, Deerfield, IL, USA). The assay had a coefficient of variation of 4.9% at 350 U/L.

2.9. Statistics

Differences between lycopene supplemented and unsupplemented groups were analysed using paired *t* tests and differences between different concentrations of LDH and lycopene uptake were analysed using analysis of variances using Graphpad software (Graphpad Prism 4, San Diego, CA, USA). *P* values less than .05 were considered as statistically significant.

3. Results

Visual inspection of the cells after 24 h confirmed that lycopene enrichment with all concentrations of liposomes killed Calu-3 cells (Fig. 1). Cells cocultured with 0.5% and 1% THF/lycopene (v/v) appeared viable; however, 2% THF/lycopene (v/v) appeared to cause considerable cell death, and 5% THF/lycopene appeared to cause complete cell death (Fig. 1). This was confirmed by the progressive increase in LDH activity seen (Fig. 2). A small increase in LDH was even seen with THF at 0.25%. Treatment of cells with lycopene

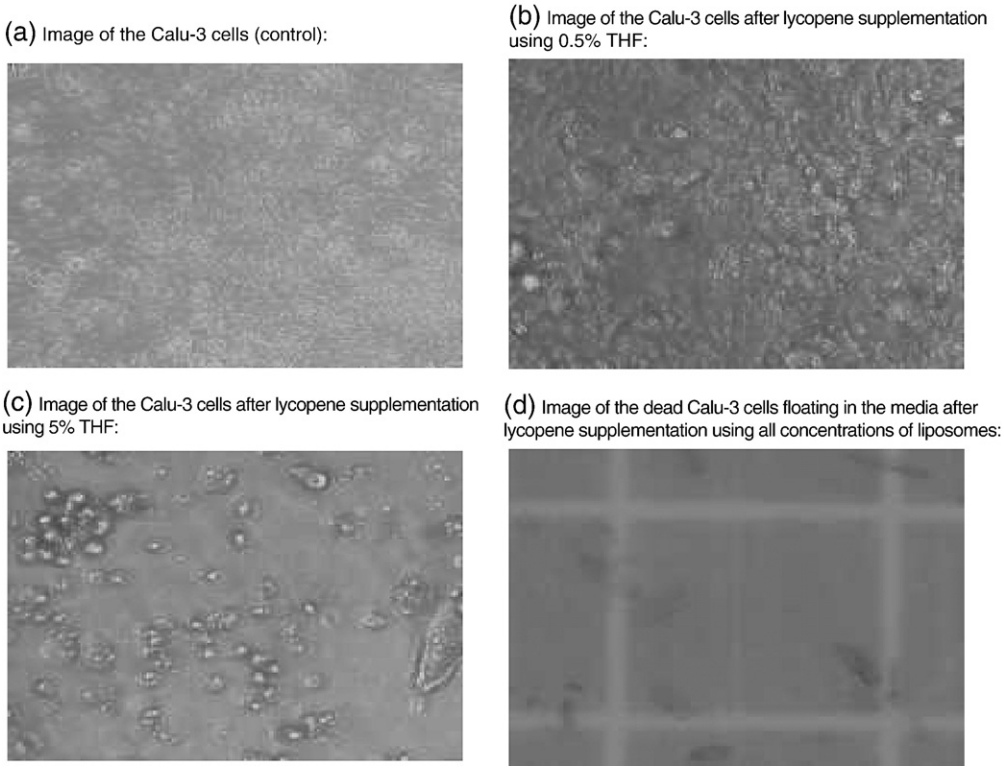

Fig. 1. Images of the Calu-3 cells before and after lycopene supplementation using THF and liposomes.

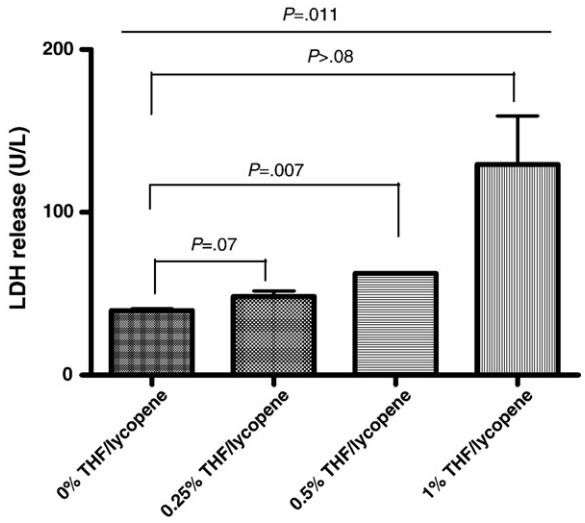

Fig. 2. Lactate dehydrogenase levels released by Calu-3 cells after 24 h incubation with 1% FCS/MEM containing different concentrations of THF/lycopene (n=3).

and THF resulted in a progressive increase in intracellular lycopene (Fig. 3). However, there was no significant difference between lycopene uptake at 1.25 or 2.5 μg/ml.

Infection with RV-43 failed to induce a significant release of IL-6; however, there was significant release following infection with RV-1B and exposure of cells to LPS (Fig. 4). Cells supplemented with lycopene showed a significant reduction in IL-6 release following infection with RV-1B and exposure to LPS (Fig. 4). Infection with RV-43, RV-1B and treatment with LPS all failed to significantly increase IL-8 release (data not shown). Infection with RV-43, RV-1B as well as LPS exposure led to a significant increase in IP-10 release (Fig. 5). Supplementation with lycopene led to a significant decrease in IP-10 release following RV-43 infection and LPS treatment. There appeared to also be a reduction in IP-10 release following RV-1B infection and treatment with lycopene, but this was nonsignificant (P=.13) (Fig. 5). Table 1 shows the IL-6, IL-8 and IP-10 mRNA levels (relative expression ratio %) in cells enriched with and without lycopene, before and after infection induced by RV-43, RV-1B and LPS exposure.

As shown in Fig. 6, $TCID_{50}$ results showed that lycopene supplementation significantly decreased viral replication of RV-1B by 85% (P=.025), and there was a trend to reduced RV-43 replication by 53%, which failed to reach significance (P=.18).

4. Discussion

This is the first study to assess the feasibility of enriching airway epithelial cells with lycopene and to assess its subsequent effect on the inflammatory response following RV infection or stimulation with LPS. We determined that (2.5 μg/ml) lycopene/THF (at 0.5% of media) was able to increase intracellular lycopene levels with minimal cellular toxicity. We then demonstrated that pretreatment of airway epithelial cells with lycopene reduced the release of IL-6 and IP-10 following RV-43 and RV-1B infection. Similarly, pretreatment with lycopene also reduced the release of IL-6 and IP-10 from cells

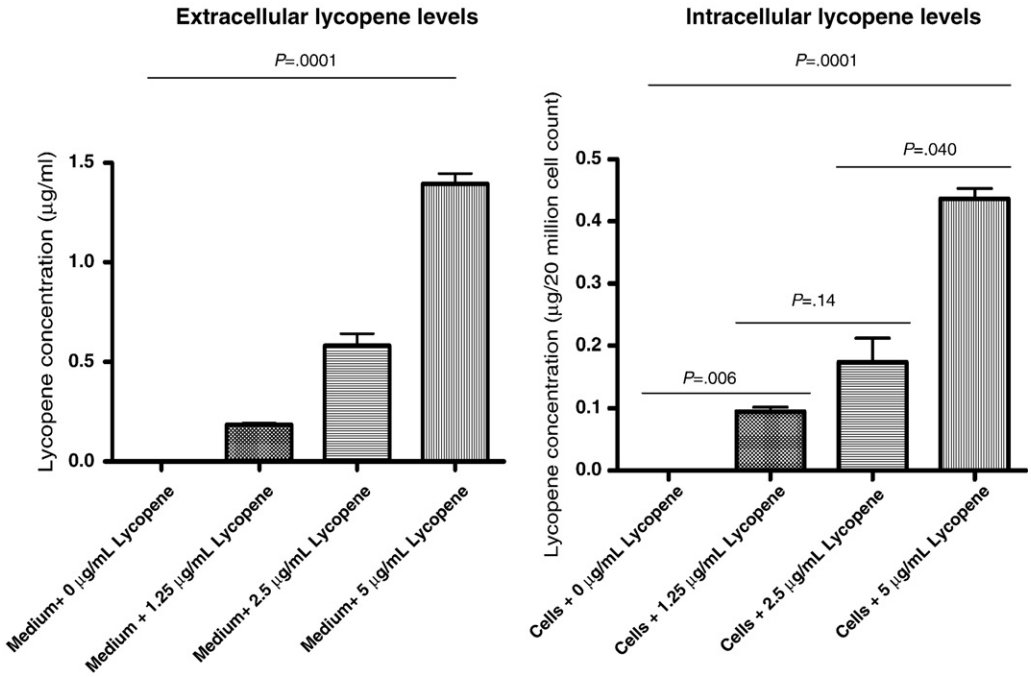

Fig. 3. Intracellular and extracellular lycopene concentrations of Calu-3 cells after 24 h incubation with different concentrations of lycopene dissolved in THF (n=3).

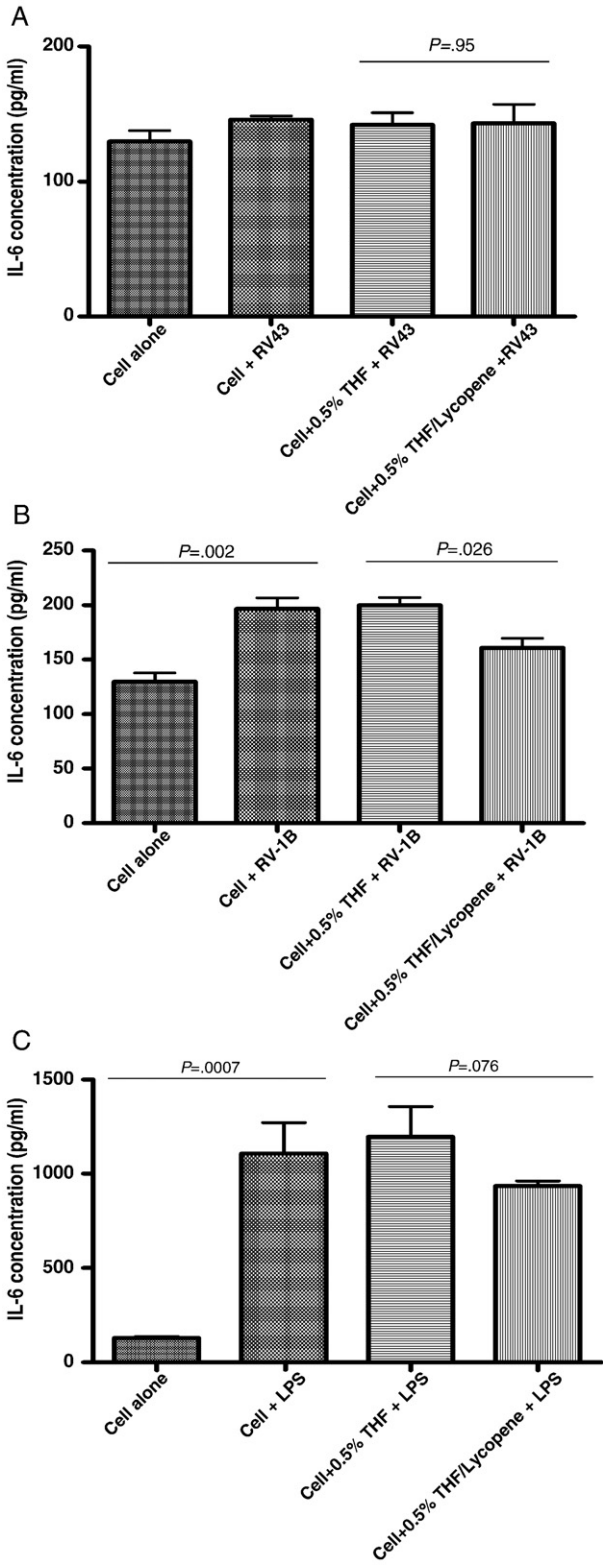

Fig. 4. IL-6 (mean±S.E.M.) released by Calu-3 cells with and without lycopene, before and after infection induced by (A) RV-43, (B) RV-1B and (C) LPS exposure.

exposed to LPS. Finally, we demonstrated that lycopene pretreatment led to a reduction in RV-1B replication and a trend to reduced RV-43 virus replication.

Although, it has been reported that lycopene enrichment of human prostate tumour cells, human lung tumour cells and

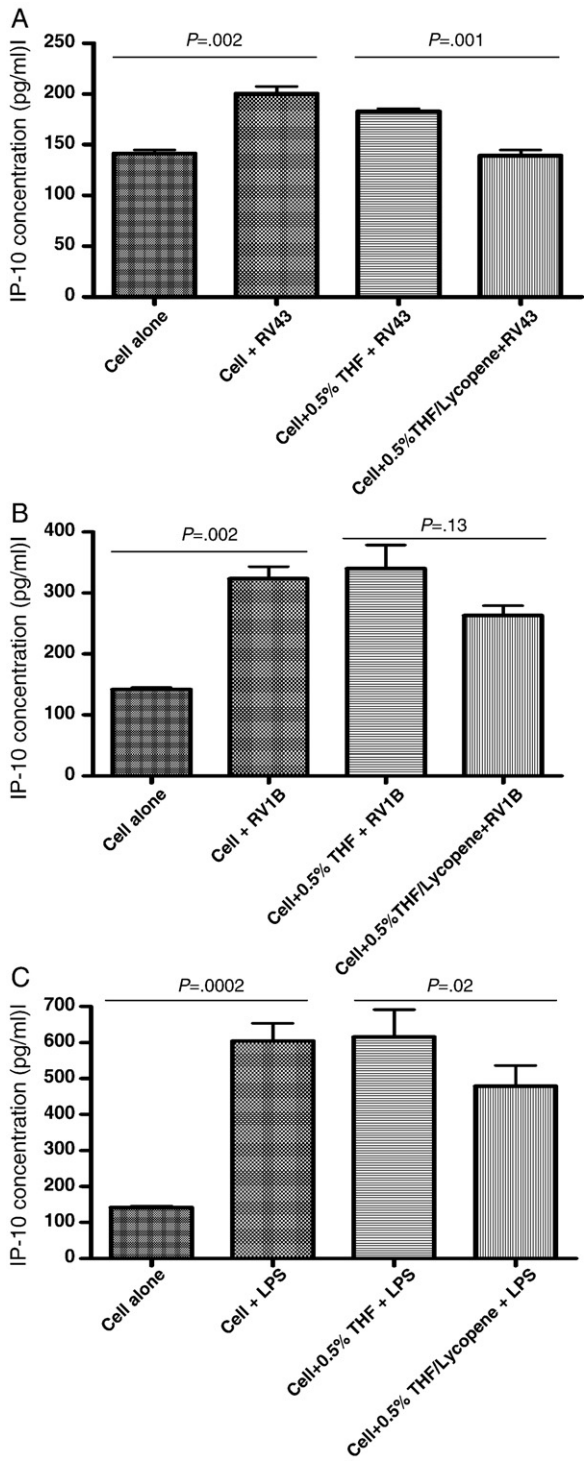

Fig. 5. IP-10 (mean±S.E.M.) released by Calu-3 cells with and without lycopene, before and after infection induced by (A) RV-43, (B) RV-1B and (C) LPS exposure.

Table 1
mRNA levels of IL-6, IL-8 and IP-10 (relative expression ratio %) in cells with and without lycopene, before and after infection induced by RV-43, RV-1B and LPS exposure

Treatments	IL-6 expression RER% (duplicate)	IL-8 expression RER% (duplicate)	IP-10 expression RER% (duplicate)
Cell alone (control)	45.5±19	97±8	1355±238
Cell+RV-43	1277±333*	110±11	5699±1571
Cell+0.5% THF+RV-43	1073±4	93±14	32467±2861
Cell+0.5% THF/lycopene +RV-43	1541±277	110±17	4917±1146†
Cell+RV-1B	2318±473*	95±7	70217±3096*
Cell+0.5% THF+RV-1B	3069±1229	92±17	98872±21973
Cell+0.5% THF/lycopene+RV-1B	341±56†	176±40	18813±4261†
Cell+LPS	7353±898*	1242±67*	364805±192314
Cell+0.5% THF+LPS	5991±1709	1458±200	115967±2314
Cell+0.5% THF/lycopene +LPS	692±128†	312±6†	104364±25819

RER%, relative expression ratio %.
* $P<.05$ compared to cell alone (control).
† $P<.05$ compared to the infected cells with no lycopene (carrier only).

human umbilical vein endothelial cells using liposomes is safe [40,42], our results showed that liposomes were toxic to cultured airway epithelial cells (Calu-3). The presence of erupted cells in the medium may be due to the presence of sodium taurocholate salt crystals (one of the liposome components), which increases the hypertonicity of the medium. A toxic effect of sodium taurocholate in cell culture experiments has previously been reported [40]. After 24 h incubation of Calu-3 cells with different concentrations of lycopene carried by THF, it was found that cells enriched with low levels of lycopene/THF (0.5% and 1% v/v) were viable, and cells enriched with 2% and 5% lycopene/THF (v/v) showed progressive loss of viability. This is in agreement with previous studies which suggest doses of THF in excess of 0.7% may be toxic to cultured cells [40]. We confirmed this observation by measuring LDH and observing a dose-dependent increase in LDH levels after incubation with higher THF/lycopene levels [49].

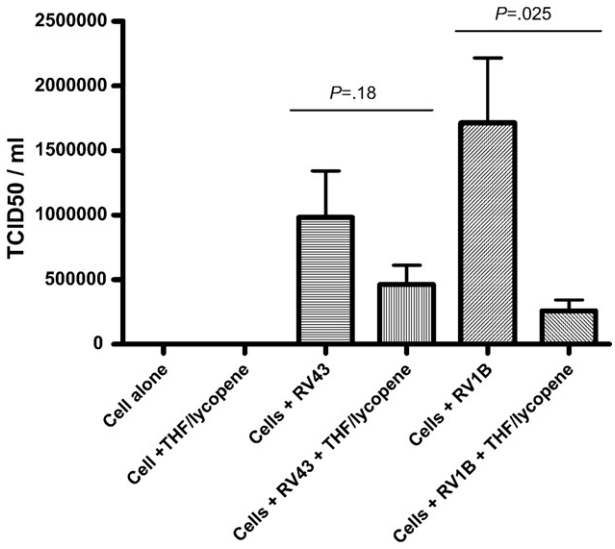

Fig. 6. $TCID_{50}$ (mean±S.D.) of RV-43 and RV-1B in the Calu-3 cells with and without lycopene (50% tissue culture infective dose).

Our data showed lycopene uptake by Calu-3 cells from the medium was also dose-dependant. In keeping with this absorption from the medium, there was decrease in the concentration of lycopene in the medium after 24 hours of exposure to the cells. This may be due to (1) cellular uptake of lycopene, (2) degradation and oxidization of lycopene in cell culture conditions, (3) changing of all-trans isomers of lycopene (in fresh medium) to *cis* isomers during incubation (which makes lycopene undetectable at its usual retention time in the detector of HPLC) and (4) destabilization of lycopene due to the use of polystyrene flasks and plates for cell incubation (compared to glass containers) [40]. It has been reported that lycopene can be delivered to the cell monolayers and its uptake after 24 h enrichment is almost twice of its uptake after 12 h, but it does not show any further increase after 48 h [50]. It has also been reported that after 48 h incubation, more than 30% of the lycopene in the medium has been reduced, and cells can obtain only 1% of the lycopene in suspension [42]. These results confirm that 24 h of incubation is most appropriate for this study. It has also been reported that high doses of lycopene may have adverse effects on cells, with lycopene concentrations more than 5 μM decreasing cell growth [50]. Therefore, in this experiment, we have optimised conditions by enriching Calu-3 cells with lycopene at a concentration of 2.5 μg/ml (that is equivalent to 4.65 μM), dissolved in 0.5% THF, for 24 h prior to exposure to RV and LPS. This amount of lycopene in the medium is almost two times higher than circulating plasma lycopene levels in healthy people, which is reported to be 0.8–1.34 μg/ml [51–53]. Therefore, the intracellular dose we achieved after our recommended method is likely to be biologically relevant to human physiologic systems. It is important to note that our study uses an experimental model of the airways. This model achieves entry of lycopene into the cells using a low concentration of THF. However, this is just a vehicle for entry of lycopene into cells in

water-based cell culture media. In the human body, lycopene is carried by natural lipoproteins into cells. In vivo, oral supplementation of lycopene is effective in increasing airway lycopene concentrations [51].

Our data showed that IL-6 and IP-10 concentrations in the media and their mRNA expression in cells increased significantly after 48 h of infection with RV-43, RV-1B as well as following LPS exposure ($P<.05$). Other researchers have found similar results after infecting cultured airway cells with rhinovirus [5,6,9]. IL-8 levels, however, were not significantly changed by virus infection or LPS exposure. This probably reflects the low MOI of RV used in the experiments [54], and higher doses may be needed to elicit release of mediators of neutrophil chemotaxis in confluent Calu-3 cells.

As an antioxidant, lycopene can reduce cellular oxidative stress [30] and acts as one of the most probable prohibitors of cytokine production via NF-κB inactivation [16,33,34]. Our results showed that lycopene significantly decreased the expression of IL-6 following RV-1B infection ($P=.026$), IL-8 after LPS exposure ($P=.01$) and IP-10 after both RV-43 infection ($P=.001$) and LPS exposure ($P=.023$). The expression of IL-6 and IL-8 proteins are mediated by NF-κB [9,14]. NF-κB has a central role in expression of IL-6 and IL-8 protein by up-regulating the transcription of a specific set of cytokine genes, in response to inflammatory stimuli [17]. Activation and translocation of NF-κB into the nucleus leads to its binding to DNA in the promoter regions of target genes, resulting in the induction of their release and the production of these proteins [55]. Similarly, inhibition of NF-κB activity can down-regulate protein expression of these cytokines [9,14]. It has also been found that other dietary antioxidants such as β-carotene and ascorbic acid can inhibit NF-κB activity in LPS-exposed cells [56,57]. This effect is attributed to their redox-based as well as non-redox-based effects on NF-κB suppression [56–59]. Lycopene has also been shown to decrease the nuclear translocation of NF-κB p65 subunit in LPS-stimulated dendritic cells [33]. Lycopene is expected to have even greater effects on the redox-based NF-κB activation, as it is a stronger inhibitor of the formation of thiobarbituric acid-reactive substances than β-carotene [30]. Thus, lycopene may reduce the release of IL-6 and IL-8 via suppression of NF-κB.

IP-10 is an important chemotactic factor for TH-1 T cells, and its release is increased following RV infection [60]. IP-10 release is also dependent upon intracellular virus replication occurring and correlates closely with RV replication [5]. It has been reported that response elements of NF-κB in the promoter region of the IP-10 gene are involved in transcriptional activation of IP-10 [61–63] and may be modified by the intracellular redox status [64]. It is also likely that virus replication, perhaps through pathways mediated by TLR-3, also induces IP-10 transcription [5]. Therefore, the decreased IP-10 levels that we have demonstrated may be a direct result of the antioxidant property of lycopene and or a direct inhibitory effect on RV replication.

We also hypothesised that lycopene would reduce RV replication in cultured airway epithelial cells. It has been reported that some nutrients such as flavones [65], vitamin A [66] and ascorbic acid [67,68] reduce the replication of some viruses. This occurs by interference with the viral replication, between viral uncoating and the initiation of viral RNA synthesis [65]. Our $TCID_{50}$ results showed that lycopene significantly decreased viral replication of RV-1B by 85% ($P<.05$). There was also a trend suggesting that lycopene decreased RV-43 replication (nonsignificant). Therefore, by decreasing viral replication, lycopene could indirectly lead to a decrease in the release of inflammatory mediators from infected cells. Decreased IP-10 production by cells is reportedly associated with low rhinovirus replication in the cells [5] that is consistent with our finding of decreased IP-10 levels due to lycopene.

In summary, we have demonstrated a method that effectively delivers and enriches lycopene into cultured human airway epithelial cells. We have also shown that presupplementation of these cells with lycopene reduces the release of IL-6 and IP-10 following RV infection and is associated with a reduction in viral replication. We also demonstrated that lycopene reduced the release of IL-6 following exposure to LPS. These results suggest that lycopene is likely to be working in two ways. Firstly, it may have a direct inhibitory effect on the formation of ROS that occur following infection and thereby reduce the activation of NF-κB via lycopene's redox-based activity. However, it is also likely that it is causing a reduction in viral replication directly and thereby reducing the release of IP-10, the mechanism by which remains unclear. This work demonstrates that the use of lycopene may potentially reduce the inflammatory effects of RV infection on the airway epithelium. The implication being that relative deficiency in lycopene or other carotenoids may be important in susceptibility to the effects of RV infection. Furthermore, manipulation of lycopene may have a preventive or treatment role in RV infection especially in the context of asthma.

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References

[1] Dreschers S, Dumitru CA, Adams C, Gulbins E. The cold case: are rhinoviruses perfectly adapted pathogens? Cell Mol Life Sci 2007;64:181–91.

[2] Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. Bmj 1993;307:982–6.

[3] Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. Bmj 1995;310:1225–9.
[4] Grunberg K, Timmers MC, de Klerk EP, Dick EC, Sterk PJ. Experimental rhinovirus 16 infection causes variable airway obstruction in subjects with atopic asthma. Am J Respir Crit Care Med 1999; 160:1375–80.
[5] Spurrell JC, Wiehler S, Zaheer RS, Sanders SP, Proud D. Human airway epithelial cells produce IP-10 (CXCL10) in vitro and in vivo upon rhinovirus infection. Am J Physiol Lung Cell Mol Physiol 2005; 289:L85–95.
[6] Subauste MC, Jacoby DB, Richards SM, Proud D. Infection of a human respiratory epithelial cell line with rhinovirus. Induction of cytokine release and modulation of susceptibility to infection by cytokine exposure. J Clin Invest 1995;96:549–57.
[7] Gern JE. Rhinovirus respiratory infections and asthma. Am J Med 2002;112(Suppl 6A):19S–27S.
[8] Yamaya M, Sasaki H. Rhinovirus and asthma. Viral Immunol 2003;16: 99–109.
[9] Zhu Z, Tang W, Ray A, Wu Y, Einarsson O, Landry ML, et al. Rhinovirus stimulation of interleukin-6 in vivo and in vitro. Evidence for nuclear factor kappa B-dependent transcriptional activation. J Clin Invest 1996;97:421–30.
[10] Kim J, Sanders SP, Siekierski ES, Casolaro V, Proud D. Role of NF-kappa B in cytokine production induced from human airway epithelial cells by rhinovirus infection. J Immunol 2000;165:3384–92.
[11] Johnston SL, Papi A, Bates PJ, Mastronarde JG, Monick MM, Hunninghake GW. Low grade rhinovirus infection induces a prolonged release of IL-8 in pulmonary epithelium. J Immunol 1998;160:6172–81.
[12] Gern JE, French DA, Grindle KA, Brockman-Schneider RA, Konno S, Busse WW. Double-stranded RNA induces the synthesis of specific chemokines by bronchial epithelial cells. Am J Respir Cell Mol Biol 2003;28:731–7.
[13] Funkhouser AW, Kang JA, Tan A, Li J, Zhou L, Abe MK, et al. Rhinovirus 16 3C protease induces interleukin-8 and granulocyte-macrophage colony-stimulating factor expression in human bronchial epithelial cells. Pediatr Res 2004;55:13–8.
[14] Zhu Z, Tang W, Gwaltney Jr JM, Wu Y, Elias JA. Rhinovirus stimulation of interleukin-8 in vivo and in vitro: role of NF-kappaB. Am J Physiol 1997;273:L814–24.
[15] Kaul P, Biagioli MC, Singh I, Turner RB. Rhinovirus-induced oxidative stress and interleukin-8 elaboration involves p47-phox but is independent of attachment to intercellular adhesion molecule-1 and viral replication. J Infect Dis 2000;181:1885–90.
[16] Biagioli MC, Kaul P, Singh I, Turner RB. The role of oxidative stress in rhinovirus induced elaboration of IL-8 by respiratory epithelial cells. Free Radic Biol Med 1999;26:454–62.
[17] Blackwell TS, Christman JW. The role of nuclear factor-kappa B in cytokine gene regulation. Am J Respir Cell Mol Biol 1997;17:3–9.
[18] Gern JE, Vrtis R, Grindle KA, Swenson C, Busse WW. Relationship of upper and lower airway cytokines to outcome of experimental rhinovirus infection. Am J Respir Crit Care Med 2000;162: 2226–31.
[19] Levandowski RA, Weaver CW, Jackson GG. Nasal-secretion leukocyte populations determined by flow cytometry during acute rhinovirus infection. J Med Virol 1988;25:423–32.
[20] Wark PA, Johnston SL, Moric I, Simpson JL, Hensley MJ, Gibson PG. Neutrophil degranulation and cell lysis is associated with clinical severity in virus-induced asthma. Eur Respir J 2002;19:68–75.
[21] Britton G. Structure and properties of carotenoids in relation to function. FASEB J 1995;9:1551–8.
[22] Roussea A, hininger I, Palazzetti S, Faaure H, Roussel A, Margaritis I. Antioxidant vitamin status in high exposure to oxidative stress in competitive athletes. Br J Nutr 2004;93:461–8.
[23] Karahan I, Atessahin A, Yilmaz S, Ceribasi A, Sakin F. Protective effect of lycopene on gentamicin-induced oxidative stress and nephrotoxicity in rats. Toxicology 2005;215:198–204.
[24] Atessahin A, Yilmaz S, Karahan I, Ceribasi AO, Karaoglu A. Effects of lycopene against cisplatin-induced nephrotoxicity and oxidative stress in rats. Toxicology 2005;212:116–23.
[25] Porrini M, Riso P, Brusamolino A, Berti C, Guarnieri S, Visioli F. Daily intake of a formulated tomato drink affects carotenoid plasma and lymphocyte concentrations and improves cellular antioxidant protection. Br J Nutr 2005;93:93–9.
[26] Rao A. Processed tomato products as a source of dietary lycopene: bioavailability and antioxidant properties. Can J Diet Pract Res 2004; 65:161–5.
[27] Tyssandier V, Feillet-Coudray C, Caris-Veyrat C, Guilland J, Coudray C, Bureau S, et al. Effect of tomato product consumption on the plasma status of antioxidant microconstituents and on the plasma total antioxidant capacity in healthy subjects. J Am Coll Nutr 2004;23: 148–56.
[28] Banhegyi G. Lycopene — a natural antioxidant. Orv Hetil 2005;146: 1621–4.
[29] Di Mascio P, Kaiser S, Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 1989;274:532–8.
[30] Stahl W, Junghans A, de Boer B, Driomina ES, Briviba K, Sies H. Carotenoid mixtures protect multilamellar liposomes against oxidative damage: synergistic effects of lycopene and lutein. FEBS Lett 1998; 427:305–8.
[31] Reifen R, Nur T, Matas Z, Halpern Z. Lycopene supplementation attenuates the inflammatory status of colitis in a rat model. Int J Vitam Nutr Res 2001;71:347–51.
[32] Hughes DA. Dietary carotenoids and human immune function. Nutrition 2001;17:823–7.
[33] Kim GY, Kim JH, Ahn SC, Lee HJ, Moon DO, Lee CM, et al. Lycopene suppresses the lipopolysaccharide-induced phenotypic and functional maturation of murine dendritic cells through inhibition of mitogen-activated protein kinases and nuclear factor-kappaB. Immunology 2004;113:203–11.
[34] Li BH, Zhang QX, Dong DJ, Lin XM. Effect of lycopene on immunity in rats with acute lung injury. Beijing Da Xue Bao 2007;39:77–82.
[35] Wood LD, Garg ML, Powell H, Gibson P. Lycopene-rich treatments modify noneosinophilic airway inflammation in asthma: proof of concept. Free Rad Res 2007 Accepted 24/10/07.
[36] Sedjo RL, Papenfuss MR, Craft NE, Giuliano AR. Effect of plasma micronutrients on clearance of oncogenic human papillomavirus (HPV) infection (United States). Cancer Causes Control 2003;14: 319–26.
[37] Sedjo RL, Roe DJ, Abrahamsen M, Harris RB, Craft N, Baldwin S, et al. Vitamin A, carotenoids, and risk of persistent oncogenic human papillomavirus infection. Cancer Epidemiol Biomarkers Prev 2002;11:876–84.
[38] Martin KR, Wu D, Meydani M. The effect of carotenoids on the expression of cell surface adhesion molecules and binding of monocytes to human aortic endothelial cells. Atherosclerosis 2000; 150:265–74.
[39] Cooney, Kappock TJt, Pung A, Bertram JS. Solubilization, cellular uptake, and activity of beta-carotene and other carotenoids as inhibitors of neoplastic transformation in cultured cells. Methods Enzymol 1993; 214:55–68.
[40] Xu X, Wang Y, Constantinou AI, Stacewicz-Sapuntzakis M, Bowen PE, van Breemen RB. Solubilization and stabilization of carotenoids using micelles: delivery of lycopene to cells in culture. Lipids 1999;34: 1031–6.
[41] Baillie AJ, Florence AT, Hume LR, Muirhead GT, Rogerson A. The preparation and properties of niosomes—nonionic surfactant vesicles. J Pharm Pharmacol 1985;37:863–8.
[42] Shahrzad S, Cadenas E, Sevanian A, Packer L. Impact of water-dispersible beadlets as a vehicle for the delivery of carotenoids to cultured cells. Biofactors 2002;16:83–91.
[43] Williams AW, Boileau TW, Clinton SK, Erdman Jr JW. beta-Carotene stability and uptake by prostate cancer cells are dependent on delivery vehicle. Nutr Cancer 2000;36:185–90.

[44] Martin KR, Loo G, Failla ML. Human lipoproteins as a vehicle for the delivery of beta-carotene and alpha-tocopherol to HepG2 cells. Proc Soc Exp Biol Med 1997;214:367–73.
[45] Barua AB, Kostic D, Olsen JA. New simplified procedures for the extraction and simultaneous high-performance liquid chromatographic analysis of retinol, tocopherols, and carotenoids in human serum. J Chrom 1993;617:257–64.
[46] Grissell TV, Powell H, Shafren DR, Boyle MJ, Hensley MJ, Jones PD, et al. Interleukin-10 gene expression in acute virus-induced asthma. Am J Respir Crit Care Med 2005;172:433–9.
[47] Reed L, Muench H. A simple method of estimating fifty percent endpoints. Am J Hyg 1938;27:493–7.
[48] Karber G. 50% end-point calculation. Arch Exp Pathol Pharmak 1931; 162:480–3.
[49] Decker T, Lohmann-Matthes ML. A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. J Immunol Methods 1988;115:61–9.
[50] Hwang ES, Bowen PE. Cell cycle arrest and induction of apoptosis by lycopene in LNCaP human prostate cancer cells. J Med Food 2004;7: 284–9.
[51] Wood LG, Garg ML, Blake RJ, Garcia-Caraballo S, Gibson PG. Airway and circulating levels of carotenoids in asthma and healthy controls. J Am Coll Nutr 2005;24:448–55.
[52] Re R, Mishra GD, Thane CW, Bates CJ. Tomato consumption and plasma lycopene concentration in people aged 65 y and over in a British national survey. Eur J Clin Nutr 2003;57:1545–54.
[53] Rissanen T, Voutilainen S, Nyyssonen K, Salonen R, Salonen JT. Low plasma lycopene concentration is associated with increased intima-media thickness of the carotid artery wall. Arterioscler Thromb Vasc Biol 2000;20:2677–81.
[54] Chen Y, Hamati E, Lee PK, Lee WM, Wachi S, Schnurr D, et al. Rhinovirus induces airway epithelial gene expression through double-stranded RNA and IFN-dependent pathways. Am J Respir Cell Mol Biol 2006;34:192–203.
[55] Schmitz ML, Baeuerle PA. The p65 subunit is responsible for the strong transcription activating potential of NF-kappa B. EMBO J 1991; 10:3805–17.
[56] Bai SK, Lee SJ, Na HJ, Ha KS, Han JA, Lee H, et al. beta-Carotene inhibits inflammatory gene expression in lipopolysaccharide-stimulated macrophages by suppressing redox-based NF-kappaB activation. Exp Mol Med 2005;37:323–34.
[57] Hartel C, Strunk T, Bucsky P, Schultz C. Effects of vitamin C on intracytoplasmic cytokine production in human whole blood monocytes and lymphocytes. Cytokine 2004;27:101–6.
[58] Bowie AG, O'Neill LA. Vitamin C inhibits NF-kappa B activation by TNF via the activation of p38 mitogen-activated protein kinase. J Immunol 2000;165:7180–8.
[59] Carcamo JM, Pedraza A, Borquez-Ojeda O, Golde DW. Vitamin C suppresses TNF alpha-induced NF kappa B activation by inhibiting I kappa B alpha phosphorylation. Biochemistry 2002;41:12995–3002.
[60] Wark PA, Bucchieri F, Johnston SL, Gibson PG, Hamilton L, Mimica J, et al. IFN-gamma-induced protein 10 is a novel biomarker of rhinovirus-induced asthma exacerbations. J Allergy Clin Immunol 2007;120:586–93.
[61] Majumder S, Zhou LZ, Chaturvedi P, Babcock G, Aras S, Ransohoff RM. p48/STAT-1alpha-containing complexes play a predominant role in induction of IFN-gamma-inducible protein, 10 kDa (IP-10) by IFN-gamma alone or in synergy with TNF-alpha. J Immunol 1998;161: 4736–44.
[62] Nazar AS, Cheng G, Shin HS, Brothers PN, Dhib-Jalbut S, Shin ML, et al. Induction of IP-10 chemokine promoter by measles virus: comparison with interferon-gamma shows the use of the same response element but with differential DNA-protein binding profiles. J Neuroimmunol 1997;77:116–27.
[63] Wu C, Ohmori Y, Bandyopadhyay S, Sen G, Hamilton T. Interferon-stimulated response element and NF kappa B sites cooperate to regulate double-stranded RNA-induced transcription of the IP-10 gene. J Interferon Res 1994;14:357–63.
[64] Conner EM, Grisham MB. Inflammation, free radicals, and antioxidants. Nutrition 1996;12:274–7.
[65] Ishitsuka H, Ohsawa C, Ohiwa T, Umeda I, Suhara Y. Antipicornavirus flavone Ro 09-0179. Antimicrob Agents Chemother 1982;22:611–6.
[66] Russell JK, Blalock JE. Vitamin A inhibition of polyoma virus replication. Biochem Biophys Res Commun 1984;122:851–8.
[67] Madhusudana SN, Shamsundar R, Seetharaman S. In vitro inactivation of the rabies virus by ascorbic acid. Int J Infect Dis 2004;8:21–5.
[68] Bissell MJ, Hatie C, Farson DA, Schwarz RI, Soo WJ. Ascorbic acid inhibits replication and infectivity of avian RNA tumor virus. Proc Natl Acad Sci U S A 1980;77:2711–5.