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Immunomodulatory Effect of Influenza Virus Infection

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ABSTRACT

Herbal ginseng medication has reported beneficial effects on human health development. We examined whether Red Ginseng Extract (RGE) preventively affects in vivo and in vitro influenza A virus infection. RGE was found to enhance the survival of human influenza virus epithelial cells. In addition, the RGE therapy was possibly partially reduced by interaction with reactive flu influenza oxygen forming molecules, due to the expression of pro-inflammatory genes (IL-6 , IL-8). Mice with RGE have had many long-term oral administration of immunomodulatory effects, such as enhancing IFN-α-cell antiviral development after infection with flu A. RGE administration in mice also stopped inflammatory cells from penetration into the bronchial lumens. Consequently, RGE may have the potential beneficial effects of several immunomodulatory roles on influenza A viral infections.

1. Introduction

Influenza A virus is a major pathogen in the respiratory tract causing substantial morbidity and mortality seasonal epidemics and pandemics. Different influenza A Hemagglutinin (HA) and Neuraminidase (NA) viruses with various combinations were identified[1]. Influenza disease vaccination has been known to be an successful process. Current vaccines do not, however, offer much security if new strains of antigenicity occur such as the recent H1N1 pandemic outbreak of 2009[2,3]. It is therefore highly important to find a preventive measure that would protect against an unpredictable strain of influenza. Although the mechanisms used to cause influenza virus infection continue to be unclear, it is probable that multiple mechanisms are involved in the influenza disease process. It was suggested that influenza A virus output of reactive oxygen (ROS) species helps promote the development of cytokines / chemokines, epithelial cell death and pulmonary damage by irregular signal transduction routes[4–7]. Such findings indicated that influenza-induced epithelis dysfunction can be regulated by hypercytokinemia and epithelial cell apophtosis. The effects of new anti-inflammatory agents may also be accompanied by medicinal herbs [8,9]. Panax ginseng is one of the herbal medicines most commonly used. The host immune regime has been improved by activating natural killer cells, T-cells, B-cells and dendritic-dependent immune system responses[10]. Panax ginseng has also been noted. Finds were shown to prevent OOS-induced oxidative stress and to modulate antioximum protection systems by whole ginseng extracts and ginseng components[11,12]. In our preceding research, mice with ginseng extracts have been found to have an advantage in survival against influenza A infection [13,14]. Nevertheless, it is still largely unknown how Ginseng extracts can play a role in giving in vitro and in vivo defense against influenza A virus infection. The effects of RGE on the cell viability, cytokine expression and cellular oxidative stress on human epithelial influenza cells were investigated in this study. In addition, we assessed RGE oral influenza A virus infection in the mouse model. Potential immunomodulatory functions

A multifactor hormone that controls immune reactions to functions that extend beyond its traditional purpose in calcium homeostasis [1, 2] is commonly referred to as vitamin D or 1.25-dihydroxyvitamin D (1,25(OH)2D). Prior studies demonstrated the vitamin D-receptor (VDR), the expression of all known biological effects of vitamin D associated with the effector cells, mainly peripheral blutaries, and the metabolization of the active form of vitamin D, 1.25 dihydroxyvitamin D[3, 4]. This suggests that in the conditions of an immune response to infection vitamin D may be a significant element[5]. In addition, several studies have shown the role of vitamin D in immune modulation[6]. The 25- hydroxy vitamin D; an inactive form of vitamin D, can be converted to 1, 25-dihydroxy vitamin D in human cells in in vitro activated cells [7]. As a consequence, 1.25-dihydroxy vitamin D developed regionally has an effect on immune cells in an autocrine or paracrine form. In the case of adapative or innate immunity responses to viral and bacterial infections, previous studies have shown that vitamin D is important. There has been a strong connection between vitamin D and respiratory diseases, like respiratory syncytial infections[8-11], TB[12], influenza[13], and other pulmonary disorders[14,15]. There is a significant association of vitamin D. An powerful association between vitamin D rates and the outbreak of pulmonary infections including influenza has also been shown by epidemiological evidence. There were also seasonal changes in serum vitamin D levels. The winter influenza occurrence has a detrimental effect on the seasonal serum levels of vitamin D.

2. Vitamin D in Immunity and Barrier Function

The Secosteroid Hormone Vitamin D is generated from 7-dehydrocholesterol in the skin and is stored and supplied in certain nutrients after ultraviolet B (UVB) exposure. It is worth highlighting some essential mechanisms involved in the host defense against infectious agents while investigating the role of vitamin D in the immunity of influenza viruses. The first level of defence, which retains tissue integrity from infectious invasion, is composed of epithelial cells covering the skin and the outer layer of the respiratory , gastrointestinal and geniturinary tract.

Furthermore, vitamin D also induces genes that are associated with gap interconnections (e.g. connection 43), tight junctions (e.g. occludin), and adhering interconnections (e.g. E-cadherin). Vitamin D has shown a strong link to a tissue special synthesis of 1,25-dihydroxy vitamin D in order to boost antifungal responses to pathogens. These effects are mediated by the VDR, also known as a subfamily 1 nuclear receptor, Member 1 (NR1I1). Their effects have been assessed by the binding effects on vitamin D. VDR is a nuclear receiver which, once the ligand is binding, is dimerised with a Retinoid X Receptor (RXR) isoform. This VDR-RXR heterodimers then bind to vitamin D-response elements (VDRE). Furthermore, VDR-RXR heterogimers can be substituted for nuclear T-cell factors, which ultimately lead to cytokine-related genes such as interleukin (IL)-2 suppression (Fig . 1). Proliferation, leading to lower interferon (IFN)-μ and IL-2 development. Reduced IFN-α development can result in impaired dendritic-cell (DC) antigen formation, resulting in reduced lymphocyte proliferation. Vitamin D increases the expression of Th2 associated cytokines, such as IL-4, which polarize the immune response of cells to Th2 phennotype, as opposed to T helper 1 (Th1) cells. In addition, the active form of vitamin D has been reported to modulate innate immune responses. Pathogens including molecular patterns (PAMPs), such as viral proteins and nucleic acids, are the most important components of innate immune responses. Stimulating TLRs induce cytokine, antimicrobial peptide, and reactive oxygen (ROS) development following ligation.

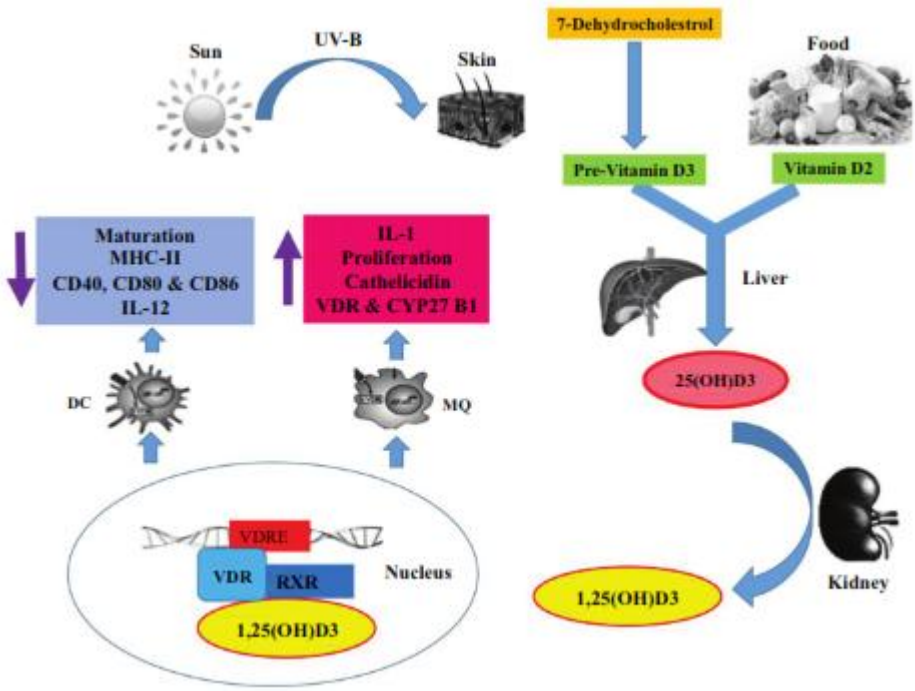

Fig. (1). Metabolism and biological effect of 1α;25(OH)2D3. Vitamin D is either supplied from dietary sources or skin surface production following UVR. The 25 hydroxylation in the liver leads to production of 25(OH)2D3 (low activity); which is converted to 1;25(OH)2D3 in kidney.

It is noteworthy that VDR induction can affect the expression of several TLRs. For example , vitamin D in epidermal keratinocytes and monocytes stimulating the expression of CD14, the co-receptor for TLR4. The activation of TLR2 in macrophages contributes to the up-regulation for cytochrome P450 family 27 subfamily B member 1 (CYP27B1 1α-hydroxylate). The association between 1,25(OH)2D and the expression of antimicrobial pectides in connection with TLR is significant. The potential chemo-attractor beta defensin 2 (HBD2) is up-regulated by 1,25-dihydric vitamin D for polymorphonuclear cells and monocytes, but vitamin D is insufficient to effectively activate mono-cytes. Human cathelicidin is another antimicrobial peptide caused by TLR1/2 activation in several immune cellulars, an endogenous antibacterial Peptide also known as Cationic host defense peptides. Propeptide hCAP18 cleavage can lead to the production of LL-37 active antimicrobial cathelicidin. Other types of immune systems, including natural killer (NK), B cells and monocytes, express hCAP18 in addition to polymorphonuclear cells reveal that this propeptide is a significant part of the immune response to infections. In this context, a number of types of immune cells are highly cathelicidal because of the vDR response element in response to 1,25(OH)2D. Cellular expression of 1αhydroxylase induces cathhelicidin expression by keratinocytes and macrophages. In the absence of 1,25(OH)2D, VDR or 1-alpha-hydroxylase, it has been recently demonstrated that macrophagic and keratinocytes have considerably reduced their ability to produce cathelicidin. Cathelicidin peptide LL-37 is also antiviral to a number of respiratory viruses. Overall, cathelicidin LL-37 is produced more by VDR overexpression in human alveolar macrophages that have anti viral effects on influenza infection.

3. Vitamin D in Influenza Infection

One of the mechanisms that could explain anti-viral activity of vitamin D in influenza infection is to upregulate the role of the virus in cathelicidin expression. A further mechanism may be a that vitamin D metabolite production of pro-inflammatory cytokines of 1,25(OH)2D. Therefore, it is important to note that the mortality rate among young people is higher in comparison with children and the elderly following the H1N1 pandemic

(1918 to 19). This may be because young adults have a robust immune system that makes them susceptible to open inflammatory immune responses. Both H1N1 and H5N1 influenza virus serotypes have been shown to cause an immune response cell-mediated type Th1. Proinflammatory cytokines, including IL-6 and Tumor necrosis factor (TNF)-α, are released in this form of immune response, causing disease severity to worsen. Evidence showing that the influenza-related inflammatory responses of 1,25-dihydroxy vitamin D can be modulated. In addition, 1.25(OH)2D inhibits the distinction between Th1 cells, thereby skewing Th1 / Th2 to a Th2 response, leading to inflammatory responses caused by influenza being suppressed (Fig . 3).

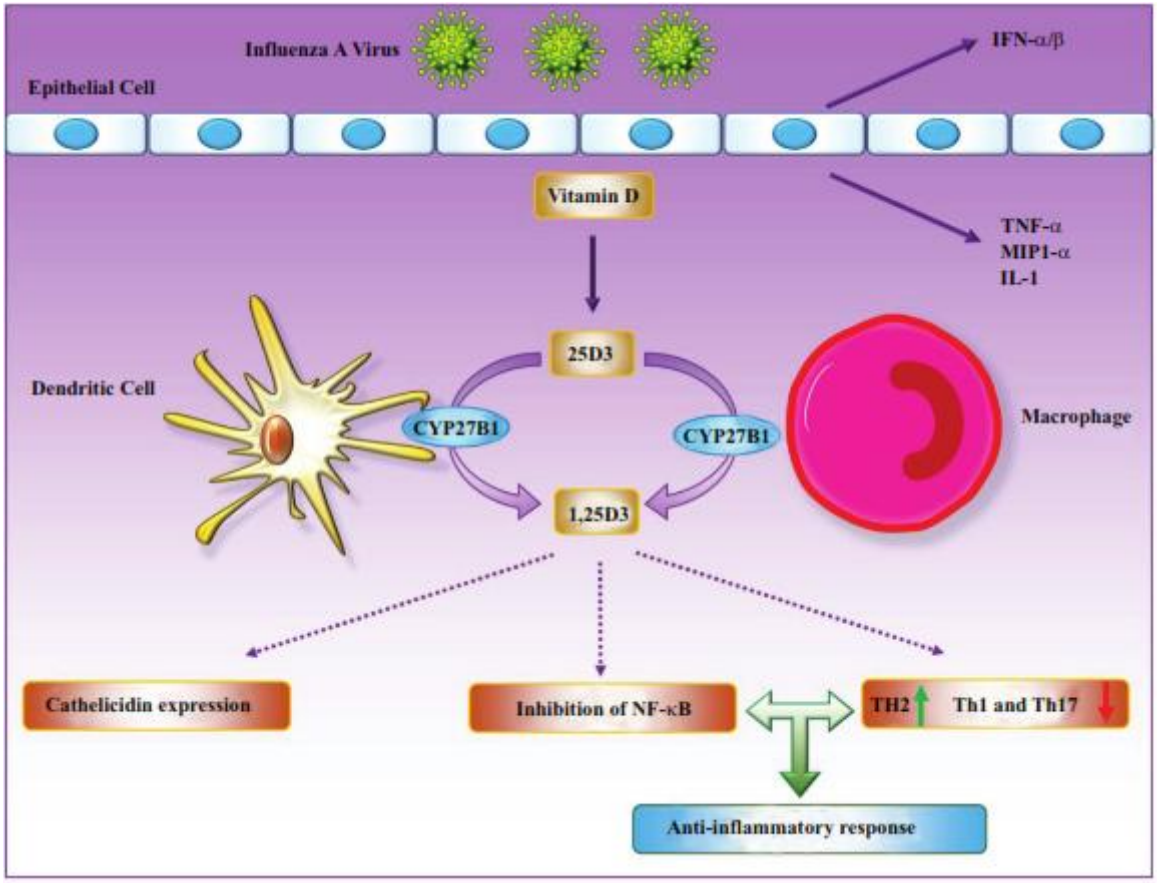

Fig. (2). The ability of Vitamin D to promote anti-influenza responses. Macrophages and epithelial cells of the pulmonary tract are the main targets of influenza A virus invasion

4. Promoting Innate Immune Responses by Production of Antimicrobial Peptides

Vitamin D can directly link influenza infection with vitamin D inducing overexpression of cathelicidin. The actions of Cathelicidin LL-37 are clear and a wide range of immunomodulatory properties including regulation of the function of neutrophils, death from infected epithelials and neutrophils, activation of autophages in infected macrophages, as well as the promotion of DCs. The result of influenza infection in humans and mice may have a significant effect on most other innate immunity processes. Human and mouse catholicide 's potent anti-influenza activity has been demonstrated in vitro and in vivo. This anti-microbial peptide, for example, protects mice against infection with influenza virus. Barlow and others have indicated that anti viral activities of murine and human cathelicidin (specific medication for the influenza virus (mCRAMP and LL-37) are comparable to zanamivir, a disease-intensive and viral load-decreasing drug. LL-37 may directly act on the influenza virus instead of receptor-based mechanisms as proposed in vitro and in vivo experiments. Cathelicidin LL-37 treatment of infected influenza mouse decreases the levels of pro-inflammatory cytokines in the lungs, compared to infected catholicidin-free mouse. Leukotriene B4 (LTB4) over-expression of LL-37 is linked to influenza enhancement A mice infection. Influenza-related inflammatory responses and reactions to the IL-1β are modulated by cathhelicidins. Interestingly, IL-1β has been reported as an essential component of host immunity in MRI. A Study by Shweta et al. also confirmed that it was mainly direct effects of the virus that LL-37 could inhibit influenza A virus strains. In comparison to human neutrophil defensa(HNP) and collectin, viral aggregation was not induced by LL-37, and cathelicidin was shown to be a clear disorder target for the lipid virus envelope. The hemagglutinin (HA) function of phil82 or PR-8 influenza A strains was not inhibited by LL-37 and defensins determined by the HA inhibition study.

5. Different Metabolic Pathways are Affected by Influenza Virus Infection

Metabolomics is a rapidly growing collection of approaches to essential constraints in the biological processes of human diseases. This is done by exploring changes in biological systems' metabolic processes in response to disease or experimental perturbation. The majority of metabolomic studies

are focused on mass spectrometry which include important data on the outcome which impact of influenza infection. The metabolomic studies have in particular told us that influenza virus infection has a metabolism effect from three main avenues: amino acid, nucleotide and lipid metabolism. Severe mortality and morbidity in influenza infection were primarily associated with inflammatory cytokines upregulation. Associations of different metabolic pathways have been identified with inflammatory cytokines. Furthermore, IL-10 has 94 metabolites including carboxychromanol, calcitriol and acylcarnitine as a cluster. It demonstrates the potential immunomodulatory functions of influenza infection with vitamin D3 (Fig . 3). These new findings suggest that the targeted inhibition and/or addition of vitamin D to cytokine profiles inhibition will stabilize metabolic pathways and defend against adverse influenza outcomes.

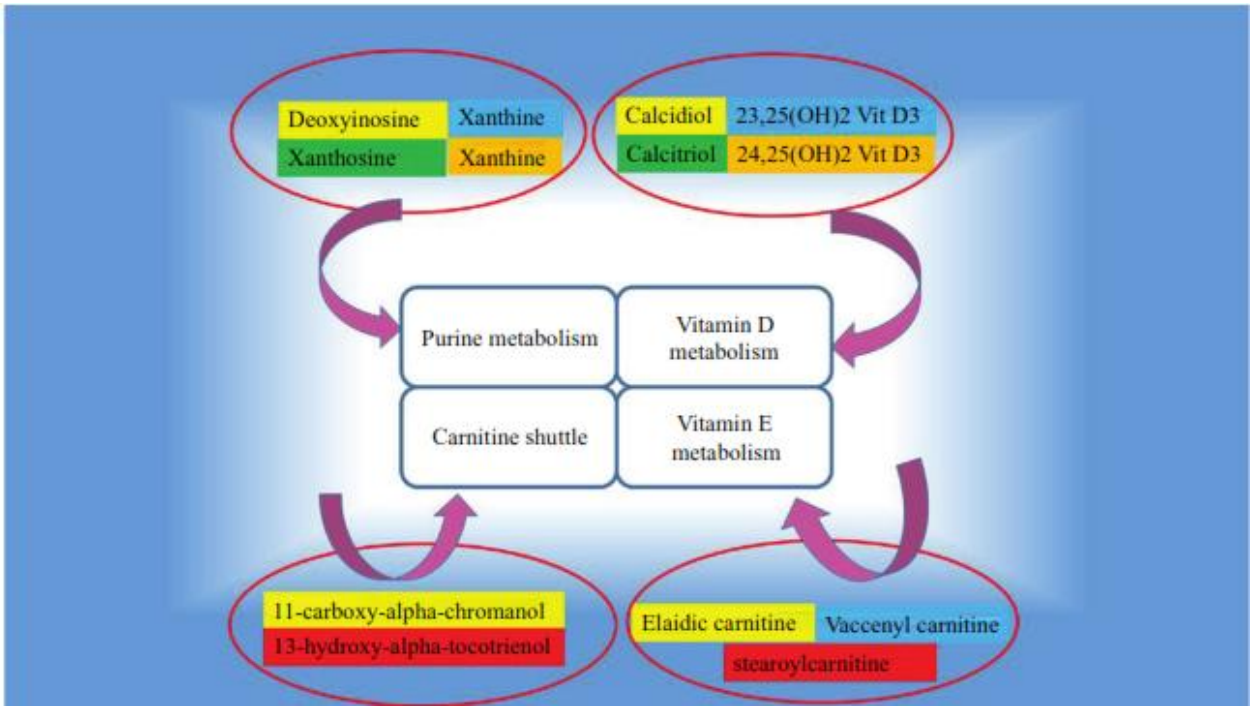

Fig. (3). Simple summery of lung metabolites affected by influenza virus infection

6. Intervention and Therapeutic Approaches

There is an insufficient number of studies examining the beneficial effect of vitamin D on influenza. Nevertheless, the therapeutic effect of vitamin D on influenza has been evaluated and several studies have shown a substantial association of vitamin D status and influenza infection persistency[13]. A recent studies cohort found that vitamin D serum levels did not correlate with a considerably less risk of influenza, while vitamin D sufficiency was substantially associated with lower risk of influenza infection in non-vaccinated individuals through retrospective subgroup studies (99, 100). [99, 100]. The research showed a substantial decrease in the incidence of acute viral pulmonary tract infection and the burden of influenza during the cold seasons in temperate areas with the maintenance of a serums concentration of 1,25(OH)2D at least 38 ng / ml. Individuals with lower serum vitamin D concentrations have twice as high a chance of serious flu infection relative to individuals with elevated serum vitamin D. UVR has vitamin-dependent and independent influenza effects. The key activation of antimicrobial peptide synths and suppression of adaptive immune responses is vitamin D and UVR in specific pathways of innate immunosynthesis. Although UVR can induce vitamin D independent effects in the skin such as activation of the pathways of IFN signage by photo-products, the systemic effects of 1,25(OH)2D are broader because of its paracrine and cellular regulation in a variety of tissues. As an measure of people's mean amount of vitamin D, William etal. analyzed the mortality rate for individuals with pneumonia in winter and summer time solar Ultraviolet B (UVB) doses. Through this study, which analyzed deaths from the large 1918-1919 influenza influenza pandemic, it has shown that in regions most exposed to solar UVB light in the USA lowest influenza-related death levels were reported, resulting in an increase in the synthesis of vitamin D. In comparison, in the area with the lowest exposure to solar UVB, the highest fatality levels were found.

It has been shown that people with a serum level of Vitamin D above 38 ng / mL recovered from the flu, but those with vitamin D levels below3 8 ng / mL took on an average of 9 days for a recovery from influenza infection. The influenza infection was recovered in average by people with Vitamin D levels lower than 38 ng / mL. A three year retrospective placebo controlled study performed in the United States included one group of ancient African American females, 800 IUs, 1.25(OH) 2D per day for 2 years and 2,000 I U Vitamin D per day for the third year. Interestingly enough, only one person in the community Vitamin D had influenza infection when the vitamin D dose was 2,000 IU / day. In the placebo category, compared to thirty out of 110 women. In addition, the placebo group showed influenza-like signs particularly in winter, while influenza-like signs were manifested independently of the season in the Vitamin D community.

7. Materials and Methods

1. Cells, Virus, Reagents

In previous studies[13,14] influenza subtypes were described as H1N1 A / PR/8/34 (A / PR8) and A / WSN/1933 viruses. Flu viruses have been cultured in 11-day old hen embryonic eggs. Allantonic fluids of egg have been harvested and processed for use at -80 ° C. Dr. Jae Hyang Lim (Center for Inflammation, Immunity & Infection, George State University) gave generous gifts to A549 cells, an alveolar human type II epithelial cell. Mice were infected with influenza virus serial dilutions and 50% lethal (LD50) dose was identified. The Korea Ginseng corporation, Daejeon, Korea has kindly supplied a concentrated form of the commercial ginseng product (RGE). Briefly, Panax 's fresh roots were washed and dried, steamed 2 to 3 hours at 100 ° C for 6 years. The roots of dried, red ginseng have been boiled for about 3 hours in 4 to 5 volumes. This preparation has been referred to as 'RGE' (roughly 36% water). GIBCO (Grand Island NY, USA) has been acquired with Fetal bovine serum (FBS), penicillin streptomycin (Penicillin) and F12 K Nutrient Mixture. The Molecular Probes (Carlsbad, CA, USA) bought dichlorodihydrofluorescein diacetate (H2DCFDA). Analytical degree is all other chemicals.

2. Cell Viability and Cytopathogenic Effect (CPE) Reduction Assays

The effect of RGE and H1N1 influenza A was calculated by bromide (MTT), which relies on the ability of viable cells to metabolically reduce MTT salt in a violet formatsan product[15], using [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] bromide research. In short, A549 cells were treated continuously with RGE from 24 hours before incubation. Cells were treated for another 48 hours with or without RGE after virus infection. The 50 µL of the MTT inventory solution (2 mg / mL) was then added to each well after different treatments in order to achieve a total response volume of 200 µL. The formazan crystals in each well were disintegrated into isopropyl alcohol following incubation at 37 ° C during 2 h and the absorption was established at 570 nm. With vehicle treated control cells, a relative percentage of cell viability was measured as 100%. As mentioned previously (16) the cytopathogenic effect reduction test (CPE) was determined. The influenza A virus infected confluent cell monolayers at the indicated infection multiplicities (MOIs). Cells were washed off non-detached virus after 1 h adsorption period. CPE caused by the virus was observed 48 hours after infection. A549 cells were treated continuously with RGE 1 day before and during infection.

3. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

In compliance with the manufacturer's instructions, the complete RNA was removed from cell pellets by using the mini kit System RNeasy (QIAGEN, Valencia, CA, USA) for the elimination of traces of contaminating DNA. A semiquantitative RT-PCR was used to calculate the sum of each interest gene as mentioned previously[17]. Briefly, cDNA Synthesis first strand utilized Total RNA (1 µg), Super Script III RT (Invitrogen, Carlsbad, CA, USA) from each sample, oligo dT primers. The mRNA rRNA was determined by the following use of oligonuculotides, IL6 sense 5′-GACAGCCACTCTTCA-3, anti-sense 5′-CATCTTGGAGGTCAGGTGTGT-3′, IL-8 sense 5′-CAGCTTTCTTGCTC-3″, anti-sense 5 ′-ACTTCCACCTCCTGC-3″, 18S rNA sense 5′-ATCCTGATCATGC-3\. RT-PCR products on 2 percent agarose gels were analyzed and bands of bromide ethidium staining showed. 18S rRNA was used as an internal control of the same mRNA expression. RT-PCR products were measured in proportional quantities by multiplying compared to funnel controls.

4. Oral Administration of Mice with RGE and Influenza

A viral infection RGE has been disbanded and purified in sterile PBS by a membrane of 0,4 µm Millipore. In animal experiments, BALB / c mice, 9 – 10 months of age (Harlan Laboratories, Indianapolis, IN, United States) had slightly isofluran anestheticated to a dose of 25 mg / kg / day orally for 30 days. RGE was administered after the test. A 0.9 to 39 mm stainless steel feeder needle with a silicone tip was used for oral administration to ensure that oesophageal and tracheic damage was prevented. Mice (n = 5 per class) were inhaled by iso fluran and intranasally infected by mouse-adapted pathogenic influenza A H1N1 virus A / PR/8/34 (1.0 LD50) for purposes of determining RGE treatment effects for H1N1 influenza A viruses. Mice have daily been tracked to report changes in weight. The Georgia State University (GSU) Institutional Animal Care and Use committee accepted all animal experiments described in this paper.

5. Cytokine Assays

At day 5 post-challenge, each lung was removed aseptically and the lung extract prepared as homogenates with frosted glass slides after challenges. The homogenates have been centrifuged for supernatants at 2000 rpm for 10 minutes. The lungs were freezed and maintained at −80 ° C until used for cytokine testing. As described before [19], Cytokine ELISA was performed. IL-6 and IFN-α kits (eBioscience, San Diego , CA , USA) were used in the manufacturer's approved procedures to detect cytokine levels in pulmonary extracts and in Bronchoalveolar (BAL) fluids.

8. Results

1. RGE Improves Viability of Human Epithelial Cells upon Infection with Influenza A Virus

The viability of human epithelial cells was not affected by RGE itself (Figure 1A). In the presence or absence of RGE, the human alveolial A549 cells were infected in specific MOIs by H1N1 influenza A virus (A / WSN/33) as a means to investigate whether red ginseng extract (R GE) will protect epithelial cells against influenza A virus infection. Dosage-dependent influenza virus A / WSN/33 (Figures 1 and 2) have been shown to cause extreme cytopathogenic effects. Human epithelial cells displayed pronounced cytopathological structure morphology, including cell rounding, and cell separation leading to cell kills, after infection from A549 cells with influenza A virus (Figure 2). The findings of previous work have shown that the apoptosis of influenza A virus-infected cells is consistent [16,22]. Curiously, human A549 epithelial cells treated with RGE have substantially decreased cytopathogens and cell mortality due to influenza A H1N1 infection (Figures 1 and 2). These protective effects were stronger at higher MOIs through RGE (Figure 1C, D). These findings therefore suggest that RGE increases the viability of human epithelial cells in infection with H1N1 influenza A virus.

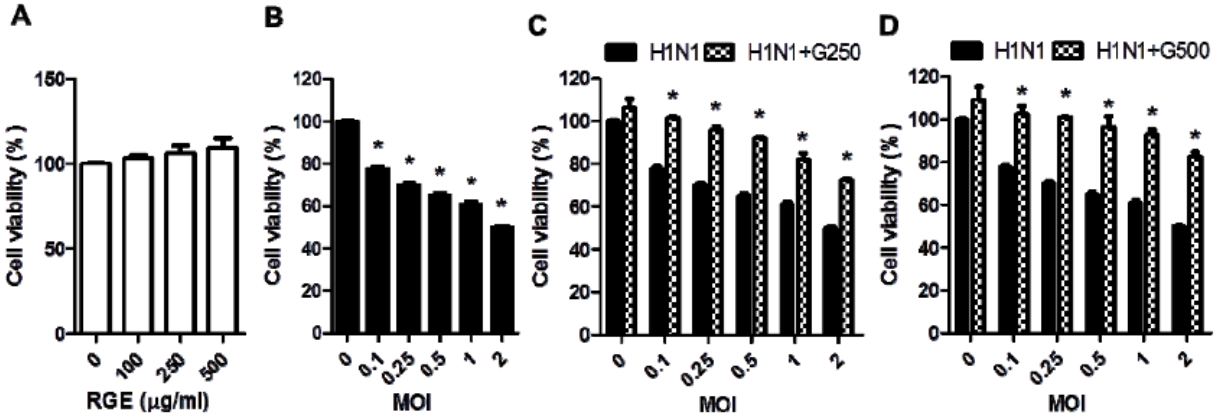

Figure 1. Influence of red ginseng extract (RGE) on H1N1 influenza A virus-induced cytopathogenic effect (CPE) formation in A549 cells. (A) Influence of different RGE concentrations on the growth of A549 cells. Values are the mean ± SEM. * p < 0.05 vs. mock control; (B) Influence of different H1N1 influenza A virus MOIs on the growth of A549 cells. Values are the mean ± SEM. * p < 0.05 vs. mock control; (C,D) Influence of different RGE concentrations on the growth of A549 cells infected with H1N1 influenza A virus at different MOIs 48 h post infection. A549 cells were continuously treated with RGE starting with 24 h pre-incubation period. Cell viability was measured by MTT assay. Values are the mean ± SEM. * p < 0.05 vs. H1N1 influenza A virus-infected group.

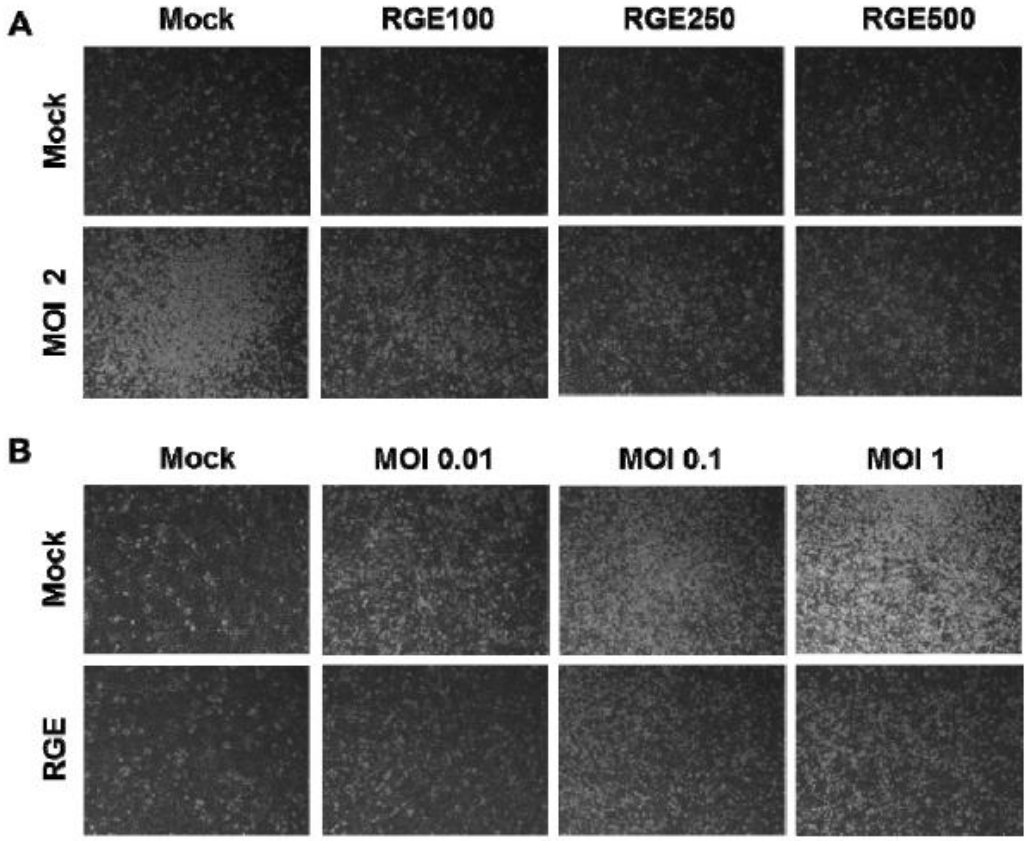

Figure 2. Representative pictures of CPE formation in A549 cells infected with H1N1 influenza A virus at different multiplicities of infection (MOIs) without or with RGE. A549 cells were mock-infected or infected with H1N1 influenza A virus at different MOIs 48 h post infection. A549 cells were continuously treated with RGE starting with 24 h pre-incubation period. (A) Influence of different RGE concentrations on A549 cells infected with H1N1 influenza A virus at a MOI of 2; (B) Influence of RGE (500 μg/mL) on A549 cells infected with H1N1 influenza A virus at different MOI from 0 to 1.

2. RGE Treatment Reduces Influenza Virus-Induced Cytokine Production

Fluin infections cause epithelial inflammation of airways and cell damage through oxidant mediation. We determined the expression of cytokine IL-6 and chemokine IL-8 for investigating the effects of RGE on inflammatory responses of influenza A epithelial cells. Influenza A 1 MOI virus was inoculated onto human epithel A549 layers in the presence or absence of the RGE procedure. Total RNA was extracted from human epithelial cells, and RT-PCR was evaluated for gene expression. In comparison to mock-treated cells in human influenza epithel cells A virus inhibits IL-6 and IL-8 expression considerably increased by influenza A induced human epithelial cells virus infected (Figure 3). IL-6 and IL-8 influenza

Influenza induced development. These findings indicate that RGE can suppress inflammatory responses to influenza A infection in lung alveolar epithelial cells.

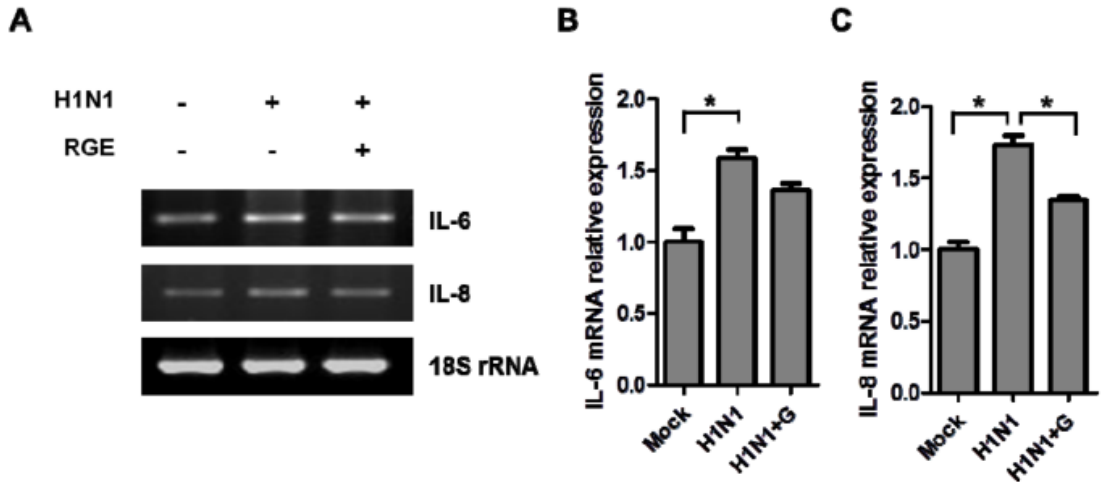

Figure 3. Influence of RGE on H1N1 influenza A virus-induced cytokine production in A549 cells. (A) Representative RT-PCR bands of cytokine mRNA expression. (B and C) The relative quantity of cytokine mRNA expression. A549 cells were mock-infected or infected with H1N1 influenza A virus. A549 cells were continuously treated with RGE (250 μg/mL) starting with 24 h pre-incubation period. After virus infection, cells were treated with or without RGE for another 48 h. Cytokine mRNA levels were determined by semiquantitative RT-PCR. Values are the mean ± SEM. * p < 0.05.

9. Discussion

Immunomodulation is considered to be one of the effective strategies for strengthening the body's viral infection defense mechanism. There is considerable evidence that ginseng has different immunomodulatory functions[10–12]. However, ginseng has not demonstrated well its potential roles in providing protection from inflammatory viral infection. Here, we showed that influenza A virus-induced cell death, pro-inflammatory cytokine expression and ROS in human epithelial cells were inhibited by RGE. Oral RGE administration to mice had immunomodulatory effects , for example IFN-μ development following influenza A. Influenza A infection. Ginseng use may therefore have a function to improve influenza A virus infection by enhancing cell survival and reducing the development of inflammatory cytokines and ROS, resulting in less inflammatory lung disease.

Pro-inflammatory hypercytokinemia has been suggested to be associated with the seriousness of human respiratory disorders by infection with virus[24]. Influenza A viruses have proved to cause expression in the epithelial airway cell of the cytokines and chemical kines including CXCL8, IL-6, CXCL10 and CCL5 (also known as RANTES)[25,26]. There seem to be several biologic and immunomodulatory effects of panax ginseng. Our preceding studies showed a high level of survival and lower levels of lung virus headaches following infection with the 2009 pandemic H1N1 infection for ginseng extract in the short-term oral treatment of mice. Mice with ginseng extract prior to infection have been found in H1N1 (A / PR/8/34) and H3N2 (A / Philippines/82) influenza influenza infections to provide survival benefits. In addition, inactivated influenza A mice, which had been co-ginseng administered, had substantially strengthened IgA and IgG specific antibodies of the influenza virus to lungs following difficult virus infections, indicating that they play a role in influenza virus mucosal adjuvant. Our data in this study show that long-term oral administration with RGE exhibited immunomodulatory functions during infection with H1N1 influenza A in naive mouse. Prior to infection, pre-therapy with RGE contributed to the defense of immunity by stimulating T and CD11c+ cells, which resulted in enhanced IFN-α development in mouse model after influenza A viral infection. RGE therapy appears to decrease the infiltration into bronchial lumens by influenza A of the inflammatory cells and lumen cells. This study thus indicates that anti-inflammatory effects on reducing Ginseng production of IL-6 and IL-8 can help prevent severe influenza A virus disease, thereby contributing to moderate survival benefit.

The pathological symptoms in humans infected by influenza viruses may be triggered by inflammatory responses. The pathogenesis of lung inflammation is characterized by oxidative stress. Influenza-infected lungs various sources of ROS have been suggested, and pulmonary epithel cells may be a source of ROS production, possibly due to the oxidative stress response caused with the influenza virus[5]. Cell oxidative damage caused by influenza virus is caused by an imbalance between the ROS production and cell defenses against antioxidants. While flu viral infection has led to a significant increase in MOD, flu viral infection has lowered gene expression of catalases. There is evidence that ROS has led to the expression of key proinflammatory mediators like cytokines and chemokines, as important influenza A regulators, causing cell physical signs from the virus. The host response to influenza infection is believed to play a key role in pathogensis with cytokines and chemokines. These impacts were related to the activation of oxidant sensitive pathways including mitogen-activated protein kinase and the nuclear factor-μb transcription factor. Our results in this study showed that influenza A infection significantly increases the rates of intracellular ROS production as well as inflammatory cytokines IL-8 and IL-6. We observed that RGE decreased the development of influenza A CPE induced by virus and prevented influenza A inflammatory gene expression induced by virus A. Although the mechanism is unknown, RGE may, at least in part, affect influenza A cellular oxidative damage caused by virus in humans in the epithelial cell line of human alveolar type 2. Our previous

findings shows that ginseng had in vitro and in vivo anti-viral influenza A growth activity [13,14]. Other antioxidants such as N-acetyl-L-cysteine (NAC), oligonol and glycyrrhizine have been described as antioxidant effects on virus replication.

10. Conclusions

In short, oxidative stress control represents a potential new pharmacology for the treatment of acute inflammation in the pulmonary system A-virus. Influenza RGE therapy Hemming A tissue-oxidative damage caused by viruses and blocked influenza induction In a human alveolar type II epithelial cell line, the virus-induced pro-inflammatory gene expression. In addition, RGE contributed to protecting immunity by enhancing IFN-μ development and partially blocking extreme penetration into the influenza A virus in an experimental mousetrain model of abundant inflammatory cells. Ginseng may, based on these results, be able to affect influenza A virus disease by antioxidative and immunomodulatory effects, although the exact underlying anti virus mechanism of ginseng is still not understood. The key factors illustrating the effects of vitamin D on influenza infection tend to be regulatory activities for vitamin D on expression of cathelicidin and development of pro-inflammatory cytokine. There is evidence of a correlation between vitamin D deficiency and influenza risk, although this is primarily based on in vitro and animal studies. The findings were positive and yet inconsistent in randomized controlled experiments and retrospective human studies complementing various types of vitamin D. Vitamin D has not been improved by vaccine immunogenicity tests on influenza vaccines. However, our review of literature indicates that treatment of individuals with influenza infections with supplements of vitamin D or cathelicidin-derived products that provide substantial protection from natural infection. In addition , Vitamin D may promote protection from seasonal flu, provided at sufficient doses. Additional clinical trials are required however in order to evaluate the efficacy of 25(OH) D supplementation in seasonal influenza infection prevention.

References

1. Uchide N, Toyoda H. Antioxidant therapy as a potential approach to severe influenza-associated complications. Molecules 2011; 16(3): 2032-52
2. Batai K, Murphy AB, Nonn L, et al. Vitamin D and immune response: Implications for prostate cancer in african americans. Front Immunol 2016; 7: 53.
3. Penna G, Adorini L. 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. J Immunol 2000; 164(5): 2405-11.
4. LeBoff MS, Yue AY, Copeland T, et al. VITAL-Bone Health: rationale and design of two ancillary studies evaluating the effects of vitamin D and/or omega-3 fatty acid supplements on incident fractures and bone health outcomes in the VITamin D and OmegA3 TriaL (VITAL). Contemp Clin Trials 2015; 41: 259-68.
5. Aranow C. Vitamin D and the immune system. J Investig Med 2011; 59(6): 881-86.
6. Baeke F, Takiishi T, Korf H, et al. Vitamin D: Modulator of the immune system. Curr Opin Pharmacol 2010; 10(4): 482-96.
7. Chen S, Sims GP, Chen XX, et al. Modulatory effects of 1,25- dihydroxyvitamin D3 on human B cell differentiation. J Immunol 2007; 179(3):1634-47.
8. Urashima M, Segawa T, Okazaki M, et al. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. Am J Clin Nutr 2010; 91(5): 1255-60.
9. Chadha MK, Fakih M, Muindi J, et al. Effect of 25-hydroxyvitamin D status on serological response to influenza vaccine in prostate cancer patients. Prostate 2011; 71(4): 368-72.
10. Cannell JJ, Vieth R, Umhau JC, et al. Epidemic influenza and vitamin D. Epidemiol Infect 2006; 134(6): 1129-40.
11. Young GA Jr., Underdahl NR, Carpenter LE. Vitamin D intake and susceptibility of mice to experimental swine influenza virus infection. Proc Soc Exp Biol Med 1949; 72(3): 695-7.
12. Vidyarani M, Selvaraj P, Jawahar MS, et al. 1, 25 Dihydroxyvitamin D3 modulated cytokine response in pulmonary tuberculosis. Cytokine 2007; 40(2): 128-34.
13. Schwalfenberg G. Vitamin D for influenza. Can Fam Physician 2015; 61(6): 507.
14. Berry DJ, Hesketh K, Power C, et al. Vitamin D status has a linear association with seasonal infections and lung function in British adults. Br J Nutr 2011; 106(9): 1433-40.
15. Kaptanis S, Chetouani L, Damle S, et al. Vitamin D supplementation for the prevention of pre-eclampsia? 'First do no harm'. BJOG 2012; 119(11): 1417; author reply 1417-1418.
16. Watkins RR, Lemonovich TL, Salata RA. An update on the association of vitamin D deficiency with common infectious diseases. Can J Physiol Pharmacol 2015; 93(5): 363-8.
17. Sabetta JR, DePetrillo P, Cipriani RJ, et al. Serum 25-hydroxyvitamin d and the incidence of acute viral respiratory tract infections in healthy adults. PLoS One 2010; 5(6): e11088.
18. Grant WB. Variations in vitamin D production could possibly explain the seasonality of childhood respiratory infections in Hawaii. Pediatr Infect Dis J 2008; 27(9): 853-3.
19. Ginde AA, Mansbach JM, Camargo CA. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the third national health and nutrition examination survey. Arch Intern Med 2009; 169(4): 384-90.
20. White JH. Vitamin D signaling, infectious diseases, and regulation of innate immunity. Infect Immun 2008; 76(9): 3837-43.
21. Philpott DJ, Girardin SE, Sansonetti PJ. Innate immune responses of epithelial cells following infection with bacterial pathogens. Curr Opin Immunol 2001; 13(4): 410-6.

