Protective Effect of Korean Red Ginseng Extract on the Infections by H1N1 and H3N2 Influenza Viruses in Mice

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ABSTRACT Ginseng has been used in humans for thousands of years and is known to have multiple biological and immunomodulatory effects. In this study, we investigated whether Korean red ginseng extract would have preventive and antiviral effects on influenza virus infection. Oral administration to mice of red ginseng extract prior to infection significantly increased survival after infection with the 2009 pandemic H1N1 virus. Daily oral treatment of vaccinated mice with red ginseng extract provided enhanced cross-protection against antigenically distinct H1N1 and H3N2 influenza viruses. Naive mice that were infected with virus mixed with red ginseng extract showed significantly enhanced protection, lower levels of lung viral titers and interleukin-6, but higher levels of interferon- γ compared with control mice having virus infections without red ginseng extract, indicating an antiviral effect of ginseng. In addition, ginseng extract exhibited inhibitory effects on the growth of influenza virus *in vitro*. This study provides evidence that intake of ginseng extract will have beneficial effects on preventing lethal infection with newly emerging influenza viruses.

KEY WORDS: • antioxidant activity • devil's claw extract • ginseng • immunomodulation • mice

INTRODUCTION

INFLUENZA IS A SERIOUS RESPIRATORY DISEASE causing seasonal epidemics that result in over 200,000 hospitalizations and 36,000 annual deaths in the United States.¹ In addition, an unexpected new pandemic influenza virus may emerge at any time. For example, the swine-origin 2009 H1N1 virus has rapidly spread throughout the world and caused a significant threat to public health. By early June 2009, the World Health Organization raised the global outbreak alert level for this novel strain to the pandemic phase, resulting in the first influenza pandemic in the 21st century. Many influenza variants have evolved to develop resistance to antiviral drugs.² Current vaccines are only effective if they are well matched with the predicted influenza virus strains

that will circulate during the next season. Indeed, from the recent experience with the 2009 pandemic H1N1 virus, it is obvious that a preventive measure that would have protective effects against the emergence of an unexpected influenza strain is highly desirable.

Ginseng is the most widely known herbal medicine and refers to the root of the plant genus *Panax*, the most common type of which is *Panax ginseng*. The major constituents of the *Panax* genus are triterpenoid glycosides or saponins (also known as ginsenosides), acid polysaccharides, and phenol and polyethylene compounds.^{3,4} Various beneficial pharmacological effects of ginseng or its components have been reported, including anticancer, anti-allergy, anti-inflammatory, antifatigue, antistress, and immunomodulatory activities.^{5–16} In clinical studies, ginseng has been shown to have beneficial effects on improving the survival and the quality of life among breast cancer patients,¹⁷ in a placebo-controlled pilot trial of cancer patients,¹⁸ in patients infected with human immunodeficiency virus,^{19,20} and on reducing the occurrence of gastric cancer.²¹ Immunomodulatory effects are among the beneficial properties attributed to

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ginseng extract. The nature of these activities and their underlying mechanisms remain largely unknown and were reported to be diverse depending on types of diseases and/or biological assessment methods: some studies demonstrated anti-inflammatory activities, while others reported pro-inflammatory effects.^{22–26}

Previous clinical studies demonstrated the beneficial effects of ginseng intake. A group of older adults who received daily oral administration of ginseng and influenza virus vaccination showed fewer cases of laboratory-confirmed influenza-like illness compared with the vaccination-only group.²⁷ Also, individuals 18–65 years of age with a history of cold symptoms in the previous year had reduced the mean number of colds by taking ginseng extract two times a day.²⁸ When taken preventatively for durations of 8–16 weeks, North American ginseng preparations were demonstrated to be effective in shortening the duration of colds or acute respiratory infections in healthy adults.²⁹ However, biological effects of ginseng on infectious disease are not well understood, and therefore further studies are important for better understanding the potential effects of ginseng.

In this study, we investigated the effects of Korean red ginseng intake on infection with the 2009 pandemic H1N1 influenza virus in naive and vaccinated mice through the oral route. Ginseng extract intake prior to infection conferred enhanced survival rates in naive mice and improved the cross-protective efficacy against H1N1 and H3N2 viruses in vaccinated mice. In addition, ginseng extract was found to have antiviral effects on influenza virus. Possible mechanisms have been investigated and discussed regarding the potential beneficial effects of ginseng on influenza virus infection.

MATERIALS AND METHODS

Cells, virus, and reagents

The 2009 pandemic swine-origin influenza A H1N1 virus (A/California/04/2009) was kindly provided by Dr. Richard Webby (St. Jude Children's Research Hospital, Memphis, TN, USA). The mouse-adapted influenza A viruses—H1N1 subtype A/PR/8/34 virus and H3N2 subtype A/Philippines/82 virus—were gifts from Dr. Huan Nguyen (University of Alabama at Birmingham, Birmingham, AL, USA). Influenza viruses were grown in 11-day-old embryonated hens' eggs and harvested as described.³⁰ Egg allantoic fluids were harvested and stored at -80°C until use. Madin–Darby canine kidney (MDCK) cells purchased from the American Type Culture Collection (Manassas, VA, USA) were maintained in Dulbecco's modified Eagle's medium and used to determine virus titers from egg allantoic fluids and mouse lung homogenates by a plaque assay.³¹ Mice were infected with serial dilutions of influenza virus, and the 50% lethal dose (LD_{50}) was determined. A/PR/8/34 virus grown in egg allantoic fluids was purified using sucrose-gradient ultracentrifugation and inactivated using formalin (0.1%) as described.³⁰ Korean red ginseng extract (ginseng extract), a concentrated form of the commercial ginseng product for human use, was kindly provided by Korea Ginseng Corp. (Daejeon, Korea). In brief, fresh

roots of *P. ginseng* that had grown for 6 years were washed, steamed at 100°C for 2–3 h, and dried. The dried red ginseng roots were boiled in 4–5 volumes of water for 3 h, and the supernatants were concentrated. This preparation was designated “ginseng extract” (approximately 36% water content).

Treatment of mice with ginseng extract and influenza virus infection

Ginseng extract was dissolved in sterile phosphate-buffered saline (PBS; pH 7.4), and filtered through a Milipore (Bedford, MA, USA) membrane (pore size, $0.4\ \mu\text{m}$). For animal experiments, 8–10-week-old BALB/c mice (Harlan Laboratories, Indianapolis, IN, USA) were lightly anesthetized by isoflurane inhalation, and then ginseng extract was orally administered in a dose ranging from 10 to 100 mg/kg of weight. Oral administration was carried out using a $0.9\ \text{mm} \times 39\ \text{mm}$ stainless steel feeding needle with a silicone tip to avoid damage to the esophagus and trachea. To determine the effects of ginseng extract treatment on influenza virus infection, mice ($n=6$ –10/group) were anesthetized by isoflurane inhalation and intranasally infected with A/California/4/2009 (2009 pandemic H1N1 virus) or A/Philippines/82 (H3N2) virus. Mice were monitored daily to record weight changes and mortality. Full details of this study and all animal experiments presented in this article were approved by the Emory University Institutional Animal Care and Use Committee (IACUC) review board and conducted under the guidelines of the Emory University IACUC. The Emory IACUC operates under the federal Animal Welfare Law (administered by the U.S. Department of Agriculture) and regulations of the Department of Health and Human Services.

Effects of ginseng extract on cross-protective efficacy by vaccination

Groups of mice ($n=10$) were intramuscularly immunized once with inactivated virus (A/PR/8/34; $1\ \mu\text{g}$). From Day 1 postvaccination, mice were administered ginseng extract (10 mg/kg) daily for 14 days. One day after the last day of ginseng treatment, vaccinated mice were challenged with the antigenically distinct A/California/4/2009 virus or H3N2 heterosubtypic A/Philippines/82 virus. Body weight changes and survival rates were daily monitored for 2–3 weeks.

Serologic test for determination of antibody responses

Influenza virus-specific antibody responses were determined by enzyme-linked immunosorbent assay as previously described.³¹ In brief, inactivated influenza virus ($4\ \mu\text{g/mL}$) was used as a coating antigen on the 96-well microtiter plates (Nunc, Rochester, NY, USA) with overnight incubation at 4°C . The wells were washed three times with PBS containing 0.05% Tween 20 and blocked with PBS containing 0.05% Tween 20 and 3% bovine serum albumin for 2 h at 37°C . Serially diluted serum samples were added and incubated for 1 h at 37°C . Then horseradish peroxidase-conjugated goat anti-mouse immunoglobulins G, G1, G2a, and G2b were used as secondary antibodies, with *o*-phenylenediamine

as the substrate. The optical density at 450 nm was read using an enzyme-linked immunosorbent assay reader.

In vivo infection with mixtures of ginseng extract and virus

Different concentrations of ginseng extract (0.5 mg or 2 mg in 25 μ L of PBS) or a PBS buffer control (25 μ L) were mixed with influenza A/California/04/2009 virus (25 μ L with 3 LD₅₀) and incubated at room temperature for 30 min. The virus and ginseng polysaccharide mixtures were intranasally administered to naive mice after anesthetization with isoflurane inhalation, and body weight changes and survival rates were monitored daily for 2–3 weeks.

Lung viral titers and cytokine assays

Lung viral titers were performed by a plaque formation assay using MDCK cells as previously described.³⁰ In brief, lungs were collected at Day 4 postinfection with A/California/4/2009 virus or a mixture of A/California/4/2009 virus and ginseng extract. Serially diluted lung extracts were added to six-well plates containing confluent MDCK cell monolayers and incubated at 37°C for 1 h. Overlay medium containing DEAE dextran, nonessential amino acids, glutamine, and trypsin was added and incubated for 2 or 3 days. After fixing with 0.25% glutaraldehyde and staining with 1% crystal violet, the plaques were counted. Cytokine enzyme-linked immunosorbent assay was performed as described previously.³² Ready-Set-Go! interferon (IFN)- γ and interleukin (IL)-6 kits (eBioscience, San Diego, CA, USA) were used for detecting cytokine levels in lung extracts following the manufacturer's recommended procedure.

Hemagglutination assay

To determine the effects of ginseng extract on influenza virus growth, MDCK cells were infected with A/California/4/2009 virus in the presence of various concentrations of ginseng extract. As a measure of influenza virus growth, culture supernatants on MDCK cells were used to determine hemagglutination activity titers as described.³³ An equal volume of 1% chicken red blood cells and culture supernatants from infected MDCK cells was incubated at room temperature for 1 h. The hemagglutination activity titers were expressed as the reciprocal of the highest dilution of culture supernatants preventing the formation of visible erythrocyte aggregates.

Statistical analysis

To determine the statistical significance, a two-tailed Student's *t* test was used when comparing two different conditions. A *P* value of <.05 was considered to be significant.

RESULTS

Ginseng extract improves survival against 2009 swine-origin H1N1 influenza virus

Ginseng products are most commonly consumed via oral intake by humans. Among other ginseng products, the Ko-

rean red ginseng extract (Korean *P. ginseng* C.A. Meyer) has been most extensively used, and its pharmacological efficacy is relatively well known. In particular, the Korean red ginseng extract used in this study was manufactured for human use. Also, its components are relatively well characterized. The major components include ginsenosides, polyacetylene, acid polysaccharides, antioxidative aromatic compounds, and insulin-like acid peptides. The Korean red ginseng contains more ginsenoside types (38 ginsenosides) than American ginseng (19 ginsenosides).³ In addition, Korean ginseng extract has been identified as containing more nonsaponin compounds, phenol compounds, acid polysaccharides, and polyethylene compounds compared with American, Sanchi, or Chinese ginseng.^{3,34}

To mimic the conventional oral intake, mice (BALB/c mice, 6–8 weeks old) were administered ginseng extract orally daily for 2 weeks. Ginseng extract suspended in PBS was administered to mice (*n*=10) at a dose of 10 mg/kg of body weight. After the end of 12 days of administration, mice were intranasally infected the next day with the H1N1 influenza A/California/2009 virus (2 $\times$ mouse LD₅₀). The survival rates of mice were monitored daily for 15 days. Mice pretreated with the 10 mg/kg dose of ginseng extract showed an 80% survival rate against a lethal dose (2 LD₅₀) of A/California/2009 virus (Fig. 1). The surviving mice were sick between Days 6 and 10 after infection as shown by losses in body weight. In the mock control mice, 20% survived and showed severe symptoms of illness, losing body weight over 25%. These results suggest that oral

FIG. 1. Ginseng extract treatment improves protection against lethal infection with the 2009 pandemic H1N1 influenza A virus: (A) changes in body weight (%) and (B) survival rates (%). Mock, mice treated with phosphate-buffered saline; Ginseng, mice treated ginseng extract. BALB/c mice (*n*=10) were treated daily with ginseng extract (10 mg/kg of weight) via the oral route. After 12 days of treatment, mice were infected with A/California/2009 virus. *Days postinfection when dead mice were excluded and only surviving mice were considered for presenting body weight changes. There is a statistically significant (*P*<.05) difference between the ginseng and mock groups in body weight changes and survival rates.

FIG. 2. Effects of different time points of ginseng treatments during infection with the 2009 pandemic H1N1 influenza A virus: (A) changes in body weight (%) and (B) survival rates (%). Mice (BALB/c mice, $n=6$) were orally administered ginseng extract (10 mg/kg) from 1 day before infection to Day 4 postinfection ($-1d+4d$) or from Day 4 to 10 postinfection ($+4d+10d$). There is no statistically significant difference between the $-1d+4d$ and $+4d+10d$ groups in body weight changes.

administration of ginseng extract can protect against the 2009 H1N1 influenza virus.

Ginseng products are in general recommended to use in healthy individuals without much scientific basis. Thus, it is of great significance to determine the ginseng's effects on host responses postinfection or when the host is not in a healthy condition. We tested the effects of different time points of ginseng treatments on influenza virus infection. Mice were orally administered ginseng extract (10 mg/kg) from either Day 1 before infection to Day 4 postinfection or Days 4–10 postinfection (Fig. 2). Ginseng treatment showed a trend of delaying body weight loss compared with the naive controls (Fig. 2A). Also, ginseng treatment started later postinfection delayed mortality (Fig. 2B). However, treating the mice with ginseng extract right before and during infection with a 2009 H1N1 virus ($2.5 LD_{50}$) was not as effective compared with the 2-week oral administration prior to infection in improving the survival rates. Therefore, ginseng extract treatment right before or postinfection provided only a minimum survival advantage.

Ginseng extract improves heterologous protective efficacy of influenza vaccines

Immunization with current inactivated influenza virus vaccine induces good protection against the same or closely related strains but not against different strains.³⁵ Consistent with this strain-specific protection, influenza vaccination in the previous season did not provide significant protection against the outbreak of a 2009 H1N1 influenza virus.³⁶

Here, we determined the effects of ginseng pretreatment on inducing heterologous or heterosubtypic protection in mice vaccinated with influenza vaccine (H1N1 A/PR8 virus). Mice were intramuscularly vaccinated with inactivated influenza A/PR/8/34 virus ($1 \mu\text{g}$ of total protein). From Day 1 postvaccination, groups of naive and vaccinated mice were treated daily with ginseng (0.2 mg per mouse per day) via the oral route for 14 days. At Day 15 postvaccination, mice were challenged with a lethal dose ($3 LD_{50}$) of a 2009 H1N1 swine-origin influenza virus (A/California/2009). Naive mice were very sick and rapidly lost body weight below 75%, and most died or had to be euthanized, showing only a 20% survival (Fig. 3). After challenge infection with an antigenically different 2009 H1N1 pandemic virus (A/California/2009), even the vaccinated mice with inactivated

A/PR8 virus vaccine (a strain isolated in 1934) showed moderately delayed body weight loss but suffered a severe loss in body weight similar to that of the naive mice control (Fig. 3A), although they showed a 60% survival rate. This low efficacy of protection against antigenically different viruses is consistent with previous studies demonstrating

FIG. 3. Oral administration with ginseng extract improves cross-protection against the 2009 pandemic H1N1 influenza A virus: (A) changes in body weight (%) and (B) survival rates (%). *Days postinfection when dead mice were excluded and only surviving mice considered for presenting body weight changes. Mice ($n=10$) intramuscularly vaccinated with inactivated virus (A/PR/8/34; $1 \mu\text{g}$) were orally administered ginseng extract (10 mg/kg) daily for 15 days prior to infection with a 2009 swine-origin influenza virus (A/California/2009) ($2.5 \times 50\%$ lethal dose). Naive, naive mice infected with virus; PR8i, vaccinated mice that were then infected with A/PR/8/34 virus; PR8i + ginseng, vaccinated mice pretreated with ginseng that were then infected with virus. There is a statistically significant ($P < .05$) difference between the PR8i + ginseng and PR8i groups in body weight changes and survival rates.

that seasonal vaccination was not effective in preventing outbreaks of 2009 pandemic viruses.^{36–38} It is notable that mice that were vaccinated with inactivated A/PR/8/34 virus followed by treatment with ginseng extract daily for 14 days showed significantly delayed and reduced body weight loss of less than 15% (Fig. 3A) and were 100% protected against the 2009 H1N1 swine-origin A/California/2009 virus (Fig. 3B). These results suggest that daily uptake of ginseng extract can improve the cross-protective efficacy of A/PR/8/34 viral vaccine against the 2009 pandemic H1N1 virus.

The intranasal route of vaccine delivery is another method widely used in humans for influenza vaccination such as live attenuated influenza virus vaccines.^{39,40} To further extend the effects of ginseng treatment on cross-protective efficacy of influenza vaccines, mice were intranasally immunized with inactivated A/PR/8/34 virus and administered ginseng extract. Naive, vaccinated-only, or vaccinated plus ginseng daily-treated mice were challenged with a lethal dose (6 LD₅₀) of H3N2 subtype influenza A/Philippines/82 virus (Fig. 4). Naive mice after infection were very sick and rapidly lost body weight below 75%, and all died. Also, mice vaccinated with inactivated A/PR/8/34 virus showed a severe weight loss and did not survive heterosubtypic challenge infection with A/Philippines/82 virus. It is important that mice that were vaccinated with an inactivated influenza virus (A/PR/8/34) and treated with ginseng daily for 14 days were 83% protected against lethal challenge

FIG. 4. Administration with ginseng extract improves heterosubtypic cross-protection of influenza virus vaccination: (A) changes in body weight (%) and (B) survival rates (%). Mice ($n=6$) intranasally vaccinated with inactivated virus (A/PR/8/34; 1 μ g) were intranasally administered ginseng extract (0.5 mg per mouse) daily for 14 days prior to infection with a heterosubtypic influenza A virus (A/Philippines/82) ($6 \times 50\%$ lethal dose). Naive, naive mice infected with virus; PR8i, mice intranasally vaccinated with inactivated A/PR/8/34 virus that were then infected with A/Philippines/82 virus; PR8i+ginseng, vaccinated mice pretreated with ginseng treatment that were then infected with A/Philippines/82 virus.

FIG. 5. Effects of ginseng administration on host humoral immune responses. Groups of mice ($n=6$) were vaccinated with inactivated A/PR/8/34 virus via the intramuscular route (IM group). A group of mice was orally administered ginseng extract (0.5 mg per mouse) daily for 14 days (IM+ginseng group). Serum antibody responses (1000 dilution) specific to A/PR/8/34 virus were determined in vaccinated mice at Day 14 postimmunization. IgG, immunoglobulin G; OD₄₅₀, optical density at 450 nm.

with the heterosubtypic A/Philippines/82 virus. Ginseng daily treatment orally did not significantly affect the immune responses to the virus after vaccination with inactivated viral antigens (Fig. 5). Therefore, these results suggest that daily administration with ginseng has a potential to improve the cross-protective vaccine efficacy of influenza vaccination.

Ginseng confers protection against influenza virus infection

To extend the potential role of ginseng extract in conferring protection against influenza virus infection, we tested the direct effects of ginseng extract on viral infection in naive mice. Mixtures of a lethal dose (1.5 LD₅₀) of the 2009 H1N1 virus (A/California/04/2009) and ginseng extract were used to infect naive mice. Naive mice that were infected with a mixture of virus and PBS buffer showed severe body weight loss, and 40% of mice survived (Fig. 6). In contrast, 100% protection was observed in naive mice that were infected with the 2009 H1N1 virus mixed with ginseng. Mice infected with the virus and 2 mg of ginseng mixture exhibited little body weight loss of approximately 5%. Also, with a dose of 0.5 mg of ginseng mixture with the virus, 100% of mice survived the viral infection, although these mice showed body weight loss up to 20%. These results indicate that ginseng exhibits antiviral effects on influenza virus infection.

In addition, lung viral titers were twofold lower in mice at Day 4 postinfection with ginseng-treated virus than those in mice infected with virus alone (Fig. 7A). Ginseng treatment of virus resulted in lower levels of the inflammatory cytokine IL-6 at Day 4 postinfection with the 2009 pandemic H1N1 virus compared with the PBS-treated virus infection (Fig. 7B). In contrast, the level of IFN- γ , a cytokine known to have an antiviral function, was found to be higher in mice infected with the ginseng-treated virus (Fig. 7C). These results suggest that ginseng might play a role in inhibiting influenza virus replication *in vivo* and increasing the antiviral cytokine IFN- γ level.

FIG. 6. Protective effects of ginseng extract against influenza virus infection. Mixtures of virus (A/California/2009) ($1.5 \times 50\%$ lethal dose) and different amounts of ginseng extract (0, 0.5, and 2 mg) were used to infect mice ($n=6$). (A) Body weight changes and (B) survival rates were monitored daily for 14 days. Naive, naive mice infected with virus in phosphate-buffered saline; Ginseng 0.5 mg, naive mice infected with a mixture of virus and 0.5 mg of ginseng extract; Ginseng 2 mg, naive mice infected with a mixture of virus and 2 mg of ginseng extract.

Ginseng inhibits growth of influenza virus *in vitro*

To better understand the protective effects of ginseng extract, we determined potential inhibitory effects of ginseng extract on influenza virus growth *in vitro*. As an indicator for viral replication, hemagglutination activity was determined in culture supernatants of MDCK cells infected with the 2009 pandemic H1N1 virus in the presence of different concentrations of ginseng extract. Virus growth was monitored by increases in hemagglutination activity. A low concentration of ginseng extract ($1 \mu\text{g/mL}$) resulted in an approximately twofold decrease in hemagglutination activity (Fig. 8). Greater inhibitory effects were observed

with increasing concentrations of ginseng extract, indicating that ginseng extract has inhibitory effects on the growth of influenza virus *in vitro*.

DISCUSSION AND CONCLUSIONS

Ginseng has been known to have various immunomodulatory functions. However, the *in vivo* effects of ginseng on influenza virus infection remain largely unknown. We investigated the effects of ginseng via oral delivery on influenza virus infection in a mouse model because oral intake of ginseng products is the most common means of consumption in healthy individuals as a nutrient supplement. Oral administration of ginseng extract to mice conferred moderate but significant resistance to infection with the 2009 pandemic H1N1 virus. The protection observed by ginseng pretreatment was not strong because ginseng-treated mice showed illness symptoms as shown by weight loss. These limited nonspecific protective effects are consistent with results from previous studies demonstrating that pretreatment with ginseng polysaccharide showed survival advantages against experimental bacterial sepsis.^{25,26} Ginseng treatment after infection showed lesser protective effects compared with those before infection. Consistent with our study, it was previously reported that saponins from ginseng extract suppressed the delayed-type hypersensitivity responses to the H1N1 influenza A/WSN virus when administered to the animals before but not after virus sensitization.⁴¹ Also, time-dependent effects of treatment with ginseng (or ginseng polysaccharides) were observed previously.^{24,26} In clinical studies, the incidence of common cold symptom complex or acute respiratory infection was also significantly lower in healthy individuals who had preventively taken ginseng.^{29,42} The results in this study support the protective effects of ginseng against influenza virus infection when taken in healthy individuals.

Most adults who consume ginseng are not immunologically naive and are either vaccinated or immune to influenza because of natural exposure. In addition, current influenza virus vaccination confers good protection against homologous or closely related strains. In this study, we found that

FIG. 7. Antiviral effects of ginseng extract on influenza virus infection *in vivo*. Lung viral titers and cytokines were analyzed in mice infected with a mixture of ginseng extract and virus (A/California/2009) ($1.5 \times 50\%$ lethal dose). (A) Lung viral titers and levels of (B) interleukin (IL)-6 and (C) interferon (IFN)- γ were determined at Day 4 postinfection with a mixture of ginseng extract and virus ($n=5$). Naive, naive mice infected with virus in phosphate-buffered saline; Ginseng, naive mice infected with a mixture of virus and 2 mg of ginseng extract. * $P < .05$ between mock and ginseng extract 0.5 mg groups as determined by Student's *t* test.

FIG. 8. Ginseng inhibits growth of influenza virus *in vitro*. Hemagglutination activity was determined in culture supernatants of Madin-Darby canine kidney cells infected with the 2009 pandemic H1N1 virus in medium containing different concentrations of ginseng extract (0, 1, 10, 100, or 500 $\mu\text{g/mL}$).

oral administration of ginseng to vaccinated mice significantly improved cross-protective efficacy by inducing 100% protection compared with 60% and 0% protection against the 2009 pandemic H1N1 virus and H3N2 subtype A/Philippines/82 virus, respectively, in the untreated vaccinated group. This would have a significant impact on benefitting public health as evidenced by the fact that seasonal vaccination could not control the spread of the 2009 pandemic virus.³⁶ Daily intake of ginseng in vaccinated mice did not induce changes in antibody responses. Consistent with this study, ginseng saponins at a dose of 10 mg/kg did not affect host immune responses, including cytotoxic T-cell activity, natural killer cell activity, or humoral antibody production in mice infected subsequently with influenza virus.⁴¹ In a clinical study, daily oral doses of ginseng before and after vaccination significantly increased antibody titers and reduced the frequency of cases with influenza cold symptoms.⁴³ In a chicken model, oral administration of ginseng stem and leaf saponin in drinking water enhanced immunity to viral diseases.⁴⁴ Further studies are needed to better understand the mechanisms of ginseng action in improving the efficacy of vaccination.

Ginseng extract when mixed with influenza virus also showed protective antiviral effects *in vivo*. Infection of mice with a mixture of influenza virus and ginseng extract resulted in lower levels of lung viral titers and IL-6 inflammatory cytokine but higher levels of IFN- γ . Ginseng saponins have been shown to have inhibitory effects on the production of stress-induced plasma IL-6 in mice.⁴⁵ In another study, acid polysaccharides of ginseng were previously found to activate macrophage cells that produce pro-inflammatory cytokines, including IFNs, tumor necrosis factor- α , IL-1 β , and IL-6.²¹ In addition, a mechanistic study demonstrated that spleen cells and peritoneal macrophage cells that were stimulated by ginseng extract produced tumor necrosis factor- α and IFN- γ inflammatory cytokines in a Toll-like receptor-4-dependent manner.²³ In contrast, ginseng was shown to attenuate lipopolysaccharide-induced pro-inflammatory mediators, including tumor necrosis factor- α and IL-6 in a macrophage cell line.²⁴ It is interesting that these immunosuppressive effects were not observed when ginseng was added after lipopolysaccharide stimula-

tion of macrophage cells.²⁴ Therefore, it is likely that ginseng may have a dual role in immunosuppressive and immunostimulatory effects.

In summary, pretreatment of mice with ginseng extract showed survival advantages against lethal challenges with the 2009 pandemic influenza virus. Oral administration of ginseng extract to vaccinated mice significantly increased the efficacy of cross-protection against the H1N1 and H3N2 influenza viruses. In addition, ginseng extract may have inhibitory activity against influenza virus growth *in vitro*, contributing to exhibiting *in vivo* protective effects on influenza virus infection. The results in this study suggest that ginseng extract seems to have multiple action mechanisms against infectious diseases, which might be beneficial when taken by healthy individuals prior to infections. Therefore, normal consumption of ginseng extract by healthy individuals would be expected to have beneficial effects on preventing influenza virus infections.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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