

Original Research

Ginseng Protects ACE2-Transgenic Mice from SARS-CoV-2 Infection

Sang Heui Seo^{1,2,*}

¹Laboratory of Influenza Research, College of Veterinary Medicine, Institute of Influenza Virus, Chungnam National University, 99 Dae-Hak Ro, Yuseong Gu, 34134 Daejeon, Republic of Korea

²Institute of Influenza Virus, Chungnam National University, 34134 Daejeon, Republic of Korea

*Correspondence: seos@cnu.ac.kr (Sang Heui Seo)

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Abstract

Background: The pandemic caused by the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) is ongoing, and despite massive vaccination campaigns, individuals continue to be infected with new SARS-CoV-2 variants. We studied the effects of ginseng, an immune-enhancing agent, on conferring immunity against SARS-CoV-2 in transgenic mice expressing the SARS-CoV-2 human angiotensin-converting enzyme 2 (ACE2) receptor. **Methods:** Human ACE2-transgenic (ACE2-tg) mice were fed ginseng extract for 180 days before they were intranasally infected with SARS-CoV-2. The mortality and morbidity were monitored for 10 days. The amount of antiviral interferon in the lung tissues was measured using enzyme-linked immunosorbent assay (ELISA) kits. **Results:** Thirty percent of the mice fed ginseng extract prior to infection survived, whereas all those that were not fed ginseng extract prior to infection died. Viral titers in the lungs were significantly lower in mice fed ginseng extract than in those not fed ginseng extract. The induction of antiviral interferon-gamma (IFN- γ) was significantly higher in the lungs of mice fed ginseng extract than in those that were not. **Conclusions:** Our data indicate that a ginseng-containing diet may enhance immunity against SARS-CoV-2 in a mouse model.

Keywords: coronavirus; SARS-CoV-2; ginseng; immunity-enhancer; antiviral activity



Graphical Abstract. Protection of mouse fed with ginseng against SARS-CoV-2.

1. Introduction

Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) belongs to a group of coronaviruses and is responsible for the global coronavirus disease-19 pandemic [1]. The first outbreak of SARS-CoV-2 was reported in late 2019 in Wuhan, China [2]. This virus has since spread rapidly worldwide. The clinical symptoms of individuals infected with SARS-CoV-2 range from asymptomatic to severe pneumonia and death [3,4].

To protect people from infection by SARS-CoV-2, vaccines using lipid nanoparticle-mRNA technology and adenovirus vectors have been developed and distributed globally. However, breakthrough infections have still been reported in fully vaccinated individuals [5–7]. SARS-CoV-

2 continues to rapidly evolve, resulting in the emergence of multiple variants. The alpha variant (B.1.1.7.), originating in the United Kingdom, showed an increased transmission rate and high mortality in infected humans [8]. Beta variants (B.1.351), originating in South Africa, have increased transmission rates, resistance to antibody therapy, and reduced vaccine efficacy [9]. The delta variant (B.1.617.2), originating in India, became dominant in most countries worldwide and showed an increased transmission rate, resistance to antibody therapy, and reduced vaccine efficacy [10,11]. Recently, a new omicron variant (B.1.1.529), originating in South Africa, contains multiple amino acid mutations in the receptor-binding domain of the spike protein, suggesting that the current vaccine may be less effective



against this variant [12,13].

Ginseng, the root of the plant *Panax ginseng* Meyer, is an herbal medicine and immune modulator native to Asia [14–16]. Ginseng has various pharmacological properties resulting from various bioactive substances, including tetracyclic triterpenoid saponins (ginsenosides), polyacetylenes, polyphenolic compounds, and acidic polysaccharides [17]. In this study, we determined whether a ginseng-containing diet could improve the symptoms of mice infected with SARS-CoV-2.

2. Materials and Methods

2.1 The Virus and Ginseng

A SARS-CoV-2 virus strain (SARS-CoV-2/human/Korea/CNUHV03/2020) was propagated in Vero cells maintained at 37 °C in a humidified 5.0% CO₂ incubator within a Biosafety Level-3 (BSL-3) facility. The facility (KCDC-08-3-03) was approved by the Korean government.

Korean ginseng (*Panax ginseng* Meyer) extract was manufactured by the Geumsan Ginseng & Herb Development Agency after purchasing a four-year-old root from Wooshin Industrial Co., Ltd. (Geumsan, Chungnam, Korea). The Korean ginseng was extracted with 70% ethyl alcohol twice at 70 °C for 6 h, concentrated in a vacuum at 70 °C to 16° Bx, and then spray-dried to obtain a powder. The Korean ginseng extract contained the following ginsenosides: Rg1 9.12 ± 0.08 mg/g, Re 15.91 ± 0.08 mg/g, Rf 4.57 ± 0.12 mg/g, Rb1 30.92 ± 0.08 mg/g, Rc 28.28 ± 0.55 mg/g, Rb2 19.23 ± 0.35 mg/g, and Rd 9.88 ± 0.26 mg/g.

2.2 Animals

Transgenic mice harboring human angiotensin-converting enzyme 2 (ACE2) [B6.Cg-Tg(K18-ACE2)2PrImn/J] (ACE2-tg mouse) were purchased from Jackson Laboratory (Bar Harbor, Maine, USA). Mice were bred in an animal facility at Chugnam National University, Korea.

2.3 Treatment of Mice with Ginseng and Infection with SARS-CoV-2

Ten five-week-old female ACE2-tg mice were fed daily with a water containing ginseng extract (50 mg/kg body weight) for 180 days before being intranasally infected with 1 × 10³ plaque-forming units (PFU) of SARS-CoV-2/human/Korea/CNUHV03/2020. The infected mice were monitored for changes in body weight and mortality for 10 days.

2.4 Measurement of Viral Titers in Lung Tissue using Real-Time Quantitative PCR

ACE2-tg mice (n = 3 per group) were fed ginseng extract and infected with SARS-CoV-2 (1 × 10³ PFU), euthanized with a high dose of isoflurane USP (Gujarat, India)

6 days post-infection, and their lung tissues were collected. Lung tissue (0.1 g) was homogenized in 1 mL of phosphate-buffered saline (PBS) (pH 7.4) and centrifuged for 5 min at 12,300 g. RNA was isolated using the RNeasy Mini Kit (QIAGEN, Hilden, Germany).

The supernatant (100 µL) was then briefly disrupted in buffer RLT (350 µL) before adding 70% ethanol (550 µL). The sample (700 µL) was transferred to an RNeasy mini spin column and spun for 15 s at 12,300 g. The flow-through was discarded, and the RW1 buffer (700 µL) was added to the spin column prior to centrifugation for 15 s at 12,300 g. The flow-through was discarded, and the RPE buffer (500 µL) was added to the spin column prior to centrifugation for 15 s at 12,300 g. The spin column was placed in a new 1.5 mL collection tube before viral RNA was eluted using 40 µL of RNase-free water.

To quantify the virus particles, we used the TaqMan real-time fluorescent PCR kit TOPreal™ One-step RT qPCR Kit (Enzynomics, Daejeon, Korea) and SARS-CoV-2 N primers and probe. The following components were mixed in a total volume of 20 µL: 5 µL of the TOPreal™ One-step RT qPCR Kit (TaqMan probe), 1 µL of 10 pmol primers containing N_Sarbeco_Forward (5'-CACATTGGCACCCGCAAT-3'), N_Sarbeco-Reverse (5'-GAGGAACGAGAAGAGGCTTG-3'), N_Sarbeco_Probe (5'-FAM-ACTTCCTCAA GGAACAACATTGCCA-3' BHQ1), 10 µL of viral RNA, and 2 µL of nuclease-free water. Real-time amplification was performed on the Rotor-Gene 6000 system (QIAGEN, Hilden, Germany) under the following reaction conditions: initial incubation at 50 °C for 30 min and 95 °C for 10 min, followed by 45 cycles of 95 °C for 5 sec and 60 °C for 30 sec. Viral titer (PFU) was calculated based on a standard curve generated using data for stock viruses with known PFU titers.

2.5 Lung Tissue Pathology

Lung tissues used for viral titration were fixed in 10% neutral-buffered formalin. The tissues were embedded in paraffin, and 0.5 µm thick tissue sections were prepared. Tissue sections were stained with hematoxylin and eosin (H&E) following the standard protocol. The stained tissue sections were observed under a DP70 light microscope (Olympus, Tokyo, Japan).

2.6 Measurement of Interferon in Lung Tissues

ACE2-tg mice (n = 3 per group) fed ginseng extract for 180 days were intranasally infected with SARS-CoV-2 (1 × 10³ PFU). Mice were euthanized with a high dose of isoflurane USP (Gujarat, India) six days post-infection before their lung tissues were collected. Lung tissue (0.1 g) was homogenized in PBS (1 mL, pH 7.4) and centrifuged for 5 min at 13,000 rpm.

The supernatants were obtained after centrifugation and used for the quantification of interferons, IFN-α, IFN-β, and IFN-γ, using a Mouse IFN-α ELISA kit (Invitrogen,

MA, USA), VeriTMKine Mouse IFN- β ELISA kit (Pestka), and Mouse IFN- γ ELISA kit (Invitrogen, MA, USA), respectively. Assays were performed according to the manufacturer's instructions. The microwell plates were washed twice with wash buffer. Assay buffer and diluted standards were added in duplicate to the wells of the plate. The sample diluent or supernatant of homogenized lung tissue was added in duplicate to the wells of the plate. The diluted biotin conjugate was added to all wells and incubated at room temperature (25 °C) for 2 h with shaking. The ELISA plates were washed four times with wash buffer. Diluted streptavidin-horseradish peroxidase was then added to each well. The plate was incubated at room temperature for 1 h on a microplate shaker, and the washing step was repeated as described above. TMB substrate was added to all the wells and incubated at room temperature for 30 min. The reaction was stopped by adding the stop solution to each microwell. The absorbance of each microwell was read using a microplate reader (Bio-Rad Laboratories, USA) at 450 nm as the primary wavelength. The amount of IFN was determined using a standard curve.

2.7 Statistical Analysis

Statistical analysis was performed using the Student's *t*-test with IBM SPSS Statistics version 20 (IBM Corp., Released 2011. Armonk, NY, USA). Statistical significance was set at $p < 0.05$.

3. Results

3.1 Protective Efficacy in Mice Fed Ginseng

ACE2-tg mice ($n = 10$ per group) were fed ginseng extract (50 mg/kg) for 180 days and infected with 1×10^3 PFU of SARS-CoV-2 virus. The infected mice were monitored for changes in body weight and mortality.

Ginseng-fed infected mice started to lose weight 6 days post-infection (p.i.), and infected mice that were not fed ginseng started to lose weight 4 days p.i. (Fig. 1A). The mean body weight percentage of ginseng-fed infected mice six days p.i. was 92.8% ($p < 0.05$) of the original mean weight prior to infection, whereas that of the infected mice that were not fed ginseng was 79.4% of the original mean weight. Uninfected mice not fed ginseng were 103.2% of their original mean weight on day 6.

When mouse mortality was observed, all infected mice not fed ginseng died within 8 days p.i. Three out of the ten ginseng-fed infected mice survived, resulting in a 30% survival rate ($p < 0.05$). All uninfected mice that were not fed ginseng survived (Fig. 1B).

3.2 Viral Titers and Pathology in the Lung Tissues of Mice Fed Ginseng

ACE2-tg mice ($n = 3$ per group) were fed ginseng extract (50 mg/kg) for 180 days and infected with 1×10^3 PFU of SARS-CoV-2 virus. The infected mice were euthanized on day 6 p.i. to measure viral titers (Fig. 2), and sam-

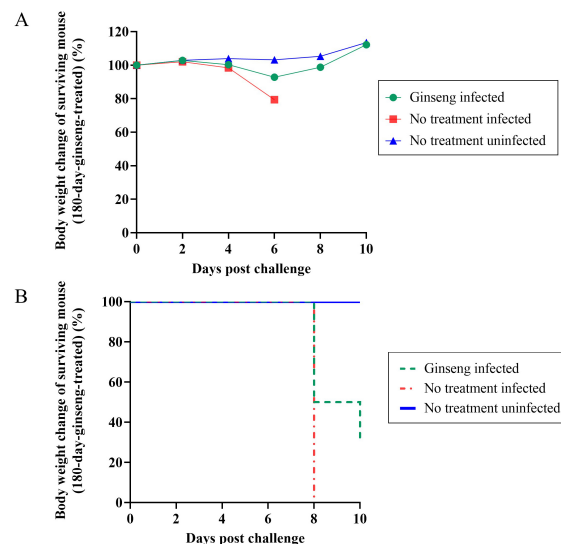


Fig. 1. Body weight change and mortality in ACE2-tg mice that were fed ginseng and challenged with SARS-CoV-2. Female ACE2-tg mice ($n = 10$ per group) fed a diet containing ginseng extract (50 mg/kg body weight) for 180 days were intranasally infected with SARS-CoV-2 (1×10^3 PFU). The infected mice were monitored for body weight change and mortality for 10 days. Statistical analysis was performed to compare ginseng-treated infected group and non-treated infected groups. (A) Body weight change in mice. (B) Mouse mortality. * $p < 0.05$.

ples were harvested for pathological staining of lung tissues (Fig. 3).

The mean viral titer was much lower in the lung tissues of ginseng-fed infected mice than in those of mice that were not fed ginseng. The mean viral titer of ginseng-fed infected mice was 1.4×10^5 PFU, while that of the infected mice that were not fed ginseng was 3.2×10^5 PFU (Fig. 2).

The lung tissues of mice were stained with hematoxylin and eosin to determine the histopathological changes caused by SARS-CoV-2 infection (Fig. 3). The lung tissue of ginseng-fed infected mice showed much milder interstitial pneumonia (Fig. 3C) than that of infected mice not fed ginseng (Fig. 3B). The lung tissues of uninfected mice not fed ginseng did not show interstitial pneumonia (Fig. 3A).

3.3 Antiviral Interferon Induction in the Lung Tissues of Mice Fed Ginseng

Interferon content in the lung tissues of infected mice euthanized on day 6 p.i. was measured (Fig. 4) to explain the possible mechanism by which ginseng protects mice from SARS-CoV-2 infection. We measured interferons (IFN- α , IFN- β , and IFN- γ), which have antiviral activity in the lungs of mice, using ELISA kits. Among the three interferons measured, IFN- β (Fig. 4B) and IFN- γ (Fig. 4C) were induced at much higher levels in the lung tissues of

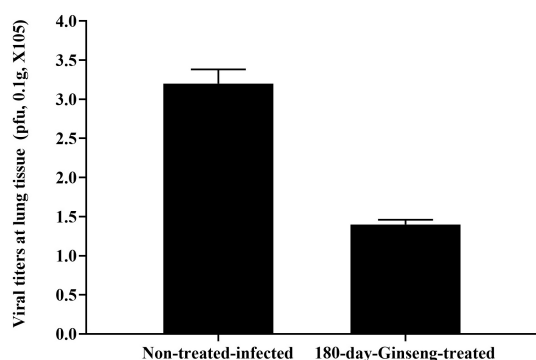


Fig. 2. Viral titers in lung tissues of ACE2-tg mice fed ginseng and challenged with SARS-CoV-2. ACE2-tg mice (n = 3 per group) fed a diet containing ginseng extract (50 mg/kg body weight) for 180 days were intranasally infected with SARS-CoV-2 (1×10^3 PFU) and euthanized on day 6 post-infection prior to the collection of lung tissues. Lung tissues were homogenized in PBS (pH 7.4), and RNA was isolated with RNeasy Mini Kit. TaqMan real-time fluorescent PCR kit with specific primers of SARS-CoV-2 was used to quantify the virus particle by PFU. Viral titer (PFU) was calculated based on a standard curve generated using data for stock viruses with known PFU titers. Statistical analysis was performed to compare ginseng-treated infected group and non-treated infected groups. * $p < 0.05$.

ginseng-fed infected mice than in infected mice that were not fed with ginseng. IFN- α was similarly induced in the lung tissues of ginseng-fed infected mice (0.1455 pg/0.1 g) and infected mice that were not fed ginseng (0.1255 pg/0.1 g) (Fig. 4A). The mean levels of IFN- β in the lung tissues of ginseng-fed infected mice and infected mice that were not fed ginseng were 0.0162 pg/0.1 g and 0.011 pg/0.1 g, respectively (Fig. 4B). The mean IFN- γ levels in the lung tissues of ginseng-fed infected mice and those not fed ginseng were 0.418 pg/0.1 g and 0.261 pg/0.1 g, respectively (Fig. 4C).

4. Discussion

In this study, we investigated whether ginseng, an immunostimulator, can boost immune responses in mice infected with SARS-CoV-2. Our results showed that feeding ACE2-tg mice with ginseng for 180 days increased their survival rate and reduced body weight loss after infection with SARS-CoV-2.

Our data showed that the survival rate of ACE-2-tg mice fed ginseng extract and subsequently infected with SARS-CoV-2 was 30%. The effect of ginseng on the survival rate (BALB/c) has also been reported in studies on influenza virus and herpes simplex virus. Mice were fed ginseng extract for two weeks prior to infection with the A/California/2009 strain of the H1N1 influenza virus (responsible for the 2009 pandemic) had an 80% survival rate [18]. Mice fed a 60-day diet containing red ginseng prior to

infection with the highly pathogenic H5N1 influenza virus had a 45% survival rate [19]. Korean red ginseng extract increased protective immunity against herpes simplex virus infection. Twenty-five percent of mice fed Korean red ginseng extract for 10 days prior to infection with herpes simplex virus survived, whereas the survival rate of infected mice that were not fed ginseng was only 10% [20].

ACE2-tg mice fed ginseng showed lower viral titers in the lungs than those not fed ginseng, which may explain their increased survival rates. In a study on the H1N1 influenza virus, lung viral titers were two-fold lower 4 days p.i. in mice that were fed ginseng than in those that were not fed ginseng prior to infection [18].

When determining the amount of antiviral interferons in lung tissue, IFN- γ levels were significantly higher in infected mice fed ginseng than in those not fed ginseng.

IFN- γ is secreted by T helper cells, cytotoxic T cells, macrophages, mucosal epithelial cells, and natural killer (NK) cells. IFN- γ acts as an important autocrine signal for professional antigen-presenting cells in the early innate immune response, as well as an important paracrine signal in the adaptive immune response [21]. IFN- γ has antiviral, immunoregulatory, and anti-tumor properties [22].

Based on our results, it seems that interferons induced by ginseng extract contributes to protecting mice from SARS-CoV-2 infection by inhibiting viral replication. More studies are needed to find out whether other mechanism helps mice to be protected from SARS-CoV-2 infection, and which ingredient of ginseng extract is important for protecting mice from SARS-CoV-2 infection.

5. Conclusions

Our study suggests that ginseng treatment can augment immune responses in ACE2-tg mice infected with SARS-CoV-2.

Author Contributions

SHS contributed to the conception, design, analysis and data interpretation, the drafting of the manuscript, and experiment work.

Ethics Approval and Consent to Participate

Animal use committee of Chungnam National University approved the animal works (202003-CNU-023).

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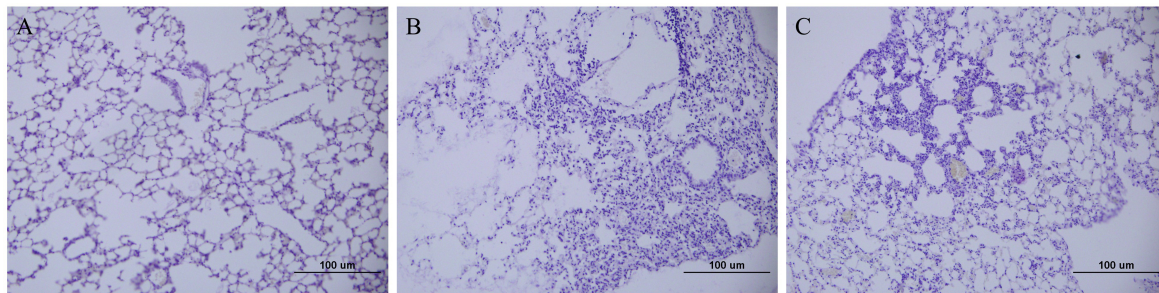


Fig. 3. Lung pathology of ACE2-tg mice fed ginseng and challenged with SARS-CoV-2. The portions of lung tissues used for viral titration in Fig. 2 were fixed in neutral-buffered formalin and were embedded in paraffin. The tissues sections (0.5 μ m thick) were stained with hematoxylin and eosin. The stained tissue sections were observed under a DP70 light microscope. (A) Lung tissue of uninfected mice not fed ginseng. (B) Lung tissue of infected mice not fed ginseng. (C) Lung tissue of ginseng-fed, infected mice.

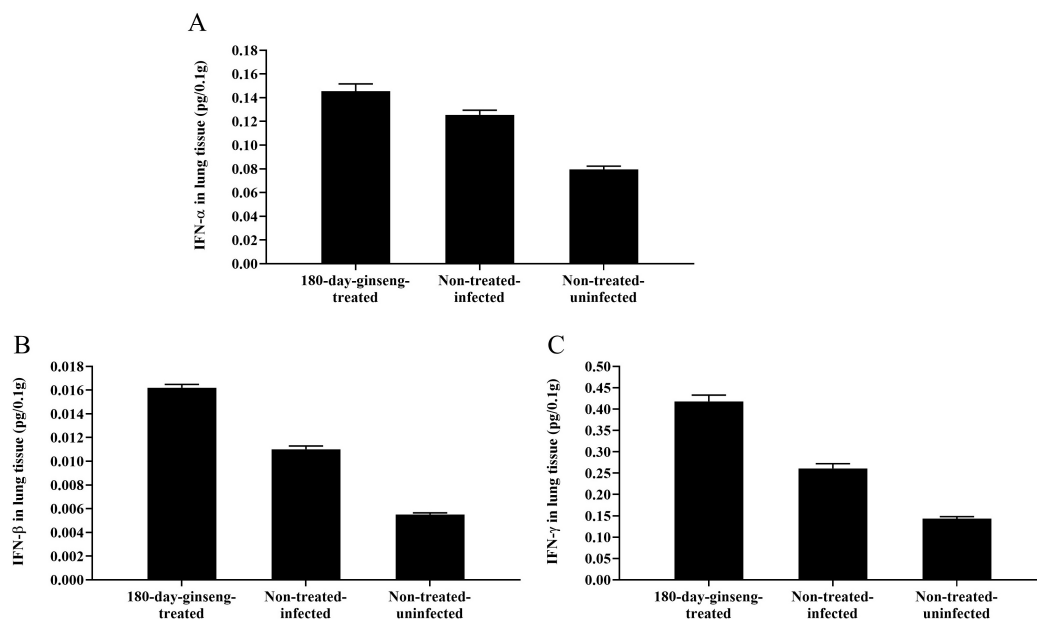


Fig. 4. Interferon amount in lung tissues of ACE2-tg mice fed ginseng and challenged with SARS-CoV-2. ACE2-tg mice ($n = 3$ per group) were fed a diet containing ginseng extract (50 mg/kg body weight) for 180 days, intranasally infected with SARS-CoV-2 (1×10^3 PFU), and euthanized on day 6 post-infection prior to the collection of lung tissues. Lung tissues were homogenized in PBS (pH 7.4) and centrifuged before supernatant collection. The amount of interferon in the supernatants of lung tissues was measured using mouse ELISA kits. Statistical analysis was performed to compare ginseng-treated infected group and non-treated infected groups. (A) IFN α . (B) IFN β . (C) IFN γ . * $p < 0.05$.

Conflict of Interest

The author declares no conflict of interest. SHS is serving as one of the Guest Editor of this journal. We declare that SHS had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to JJ.

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