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Preparation of an Inactivated Influenza Vaccine Using the Ethanol Extracts of Medical Herbs

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As seen in the COVID-19 pandemic, unexpected emergence of new viruses presents serious concern on public health. Especially, the absence of effective vaccines or antiviral drugs against emerging viruses significantly increases the severity of disease and duration of viral circulation among population. Natural products have served as a major source for safe and effective antiviral drugs. In this study, we examined the virucidal activity of medical herb extracts with a view to discover novel antiviral agents with desired levels of safety and antiviral efficacy. Ethanol extracts of ten selected medical herbs were tested for antioxidant activity and *in-vitro* cytotoxicity in various animal cell lines. Of note, the herbal extracts showed broad and potent virucidal activities against rotavirus, hepatitis A virus, and influenza A virus. The extracts of *Sorbus commixta* and *Glycyrrhiza uralensis* showed strong virucidal activities against influenza A virus. We also examined whether the extracts of *Sorbus commixta* and *Glycyrrhiza uralensis* can be used as inactivating agents to prepare an inactivated viral vaccine. In a mouse model, influenza A virus inactivated by the extracts elicited high levels of neutralizing antibodies, and the vaccination provided complete protection against lethal challenge. These results suggest that herb-derived natural products can be developed to antiviral drugs as well as inactivating agents for preparation of inactivated viral vaccines.

Key words : Antibody, antiviral drug, inactivated vaccine, medical herb, natural product

Introduction

The catastrophic outcome of current COVID-19 pandemic shows that effective antiviral drugs and vaccines are essential for the rapid control of infectious diseases. Thus far, more than 90 antiviral drugs have been approved for the treatment of only nine human-infecting viruses; HIV, HBV, HCV, HCMV, HSV, HPV, RSV, VZV, and influenza virus [10]. However, approved antiviral drugs show unsatisfactory clinical outcomes against the viral infections and frequent occurrence of resistant strains to the antiviral drugs remain a great challenge to public health [16]. Accelerated climate change increases the emergence of new viruses and cross-species viral transmission risk between animals and humans [6]. Considering that greater than 200 human viruses have been identified thus far, it is urgently required to develop safe and effective antiviral drugs. Owing to increasing possibility of emergence of hitherto unknown viruses and low efficacy of current antiviral drugs, discovery of novel antiviral agents is highly pursued.

With biosafety and various physiological activities, plant-derived natural products have historically made a great contribution to the development of prophylactic and therapeutic agents, especially for cancers and infectious diseases [3]. It is estimated that 25% of commonly used medicines include natural compounds isolated plants [22]. A growing body of literature has shown that plant-derived natural products exert antiviral activities against diverse viruses such as HIV, HSV, influenza virus, hepatitis virus, and coxsackievirus [1, 17].

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In addition, the absence of effective antiviral agents against the COVID-19 virus have led to global efforts to discover novel plant-derived natural products with antiviral activity, identifying the viral protease and RNA-dependent RNA polymerase as promising targets [7, 8, 14]. Medical herbs have long been widely used to treat infectious diseases due to multiple health beneficial effects such as direct antiviral effect and indirect immunomodulating effect [27]. Intriguingly, it has been widely recognized that plant-derived extracts show broad-spectrum antiviral activity against diverse viruses [24]. Although their precise molecular mechanisms on antiviral activity remains largely unknown, plant-derived natural products provide promising candidates for the development of improved antiviral drugs.

In this study, we examined the virucidal activities of ethanol extracts of ten selected medical herbs that have been widely used in traditional Chinese medicine. While antiviral activity encompasses preventive or therapeutic abilities against viruses, virucidal activity only refers to the capacity to inactivate viruses, thus eliminating viral infectivity. It should therefore be noted that not all antiviral compounds possess virucidal activities. Our major focus was placed on the virucidal activity, considering that virucidal compounds can be easily converted into inactivating agents for the preparation of inactivated virus vaccines. Major human-infecting viruses, influenza A virus (IAV), rotavirus (RV), and hepatitis A virus (HAV) were used as target viruses to test the virucidal activity of the extracts. In addition, the extract-treated and thus inactivated influenza A virus was used as vaccine antigens to investigate the immunogenicity and protective efficacy in a mouse model. Our results suggest that medical herb-derived natural products can be developed into antiviral drugs and inactivating agents for the preparation of inactivated viral vaccines.

Materials and Methods

Cell lines, viruses, and preparation of ethanol extracts of medical herbs

Madin-Darby canine kidney (MDCK) cells were maintained in MEM supplemented with 10% FBS. Vero, MA104, and RAW264.7 cells were maintained in DMEM supplemented with 10% FBS. HAV (HM175 strain), RV (Wa strain), and IAV (A/Puerto Rico/8/34 H1N1) were used in this study. Medical herbs were purchased from Dongwoodang pharmacy (Yeongcheon, Korea). The extracts of the herbs were prepared by ethanol extraction method. 50 g of powdered raw materials were mixed with 500 ml of 70% EtOH and extracted for 10 hr at 27℃. The extracts were filtered through filter paper and were lyophilized.

Measurement of polyphenols and antioxidative activity of extracts

Total amounts of polyphenols contained the extracts were determined using the Folin-Ciocalteu reagent, with Gallic acid as the standard. 10 μl of ethanol extracts and 200 μl of 2% Na_2CO_3 were incubated for three mins, and 10 μl of 50% Folin reagent was added for additional incubation for 30 mins, and the absorbance of the mixture was measured at 760 nm. Total amounts of polyphenol contents were determined as Gallic acid equivalents. Antioxidative activity of the extracts were measured through DPPH radical scavenging assay, as previously described [5]. Briefly, 20 μl of the ethanol extracts were incubated with 180 μl of 100 μM DPPH reagent for 30 mins, and the absorbance of the mixture was measured at 514 nm.

In vitro cytotoxicity tests by MTT assay

In vitro cytotoxicity of the extracts was measured using MA104, Vero, and MDCK cells through MTT assay. 1×10^4 cells were cultured in 96-well plates and various concentrations of the extracts (100 μl in MEM) were treated to the cells for 24 hr at 37℃. 1 mg/ml MTT reagent was added to the cells for 4 hr and 100 μl of DMSO was added to the wells for additional 15 mins. The absorbance of the mixture was measured at 540 nm. Cell viability (%) was calculated as the absorbance of sample relative to that of the negative control. Cytotoxicity test was also used to measure the virucidal activity of the extracts against RV. Ten multiplicity of infection of RV was treated with extracts for 24 hr at 37℃. The mixture was added to Vero cells for viral infection. Three days later, the cell viability was measured by MTT assay.

Measurement of virucidal activity of ethanol extracts against HAV

5×10^7 gene equivalent of HAV was incubated with various concentrations of extracts for 24 hr at 37℃, and the mixtures were added to Vero cells for viral infection. Three days later, HAV RNA in culture supernatant was isolated by QIAamp Viral RNA Mini Kit (QIAGEN). HAV RNA copies were measured by quantitative PCR with HAV-specific primers using AgPath-ID RT-PCR Reagents (ThermoFisher).

ELISA for antibody measurement

96-well plates were coated with 1×10^5 plaque forming units (PFUs) of IAV. The plates were washed with PBST buffer and blocked with 1% BSA for 1 hr. The plates were incubated with two-fold serial dilutions of sera for 1 hr. After washings, the plates were incubated with goat anti-mouse IgG polyclonal secondary antibodies conjugated with HRP (Abcam, UK) for 1 hr. The plates were washed and incubated with TMB substrate solution for 30 mins. 2*N* sulfuric acid solutions was added to stop the colorimetric reaction, and the absorbance was measured at 450 nm.

Hemagglutinin inhibition assay and microneutralization assay

For hemagglutinin inhibition (HI) assay, two-fold serial dilutions of sera (25 μl) were incubated with an equal volume of influenza viruses for 1 hr at 37℃. 50 μl of 1% chicken red blood cells was added to the wells and incubated for 1 hr at 4℃. HI antibody titers were expressed as the highest serum dilution that completely inhibited hemaggutination. For microneutralization (MN) assay, two-fold serial dilutions of sera were incubated with 100 tissue cell infectious dose 50 of IAV for 1 hr at 37℃. The mixtures were added to MDCK cells for viral infection. Three days later, cytopathic effect (CPE) of the cells was checked under microscope. MN antibody titers were expressed as the highest serum dilution that completely inhibited CPE.

Measurement of cytokine production and NO synthesis

To analyze the effect of the extracts on proinflammatory cytokine production in RAW264.7 cells, 100 μg/ml extracts and 100 ng/ml lipopolysaccharides (LPS) were co-treated to cells for 24 hr at 37℃. Secreted TNF-α, IL-1β, and IL-6 contained in the media were quantitatively determined using ELISA kits provided by the manufacturer (Abcam, UK). NO assay was used to measure the NO synthesis in RAW264.7 cell treated with the extracts. 100 μg/ml extracts and 100 ng/ml lipopolysaccharides (LPS) were co-treated to the cells for 24 hr at 37℃. The culture media were mixed with the equal volume of 40 mg/ml Griess reagent for 15 mins, and the absorbance was measured at 540 nm. NO synthesis was determined using standard curve of sodium nitrite.

Animal vaccination and infection

For mice vaccination, 6-week-old female BALB/c mice (Orient Bio Inc.) were intraperitoneally inoculated with 50 ml of formalin- or extract-treated IAV (5×10^5 PFUs). The inactivated viruses were inoculated into mice two times for prime and boost vaccinations with the interval of two weeks. Sera were collected by retro-orbital bleeding under anesthesia at two weeks after boost vaccination. For mice challenge, vaccinated mice were intranasally infected with 10 mouse lethal dose 50 of IAV (A/Puerto Rico/8/34 H1N1) at four weeks after vaccination. After challenge, body weight changes and survival rates of the mice were monitored daily for two weeks. All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the International Vaccine Institute (IACUC PN2020-022) and carried out in the ABSL-2 facility. According to guidelines for humane endpoints in animal studies, mice that lost greater than 25% of initial body weight were considered as dead and euthanized.

Statistical analysis

All values are expressed as the mean ± standard deviation (SD). A Student t-test was used to compare two groups, and the *P* value less than 0.05 was considered as statistically significant.

Results

The amounts of polyphenols and antioxidative activity of the extracts of medical herbs

For the present study, ten medical herbs were selected based on the previous study demonstrating the immune-enhancing activity of the herb extracts (Table 1) [4]. The extracts of the herbs were prepared by ethanol extraction method, and the total amounts of the extracts are shown in Table 1. It is well known that plant polyphenols have many health beneficial effects including antioxidative ability [23]. Although the direct relationship between antioxidative activity of polyphenols and their antiviral activity has not been clearly described yet, several studies have suggested that the antiviral activities of polyphenols are closely related with antioxidant and anti-inflammatory activities [21, 25]. Total amounts of polyphenols in the extracts were measured by Folin-Ciocalteu method. Among ten extracts, Lc contained the highest level of polyphenols (21.6 mg), followed by Co (15.7 mg) and Gu (12.3 mg) (Table 1). However, the antioxidative ability of Lc was not as strong as expected, yielding 33.9% of radical scavenging ability, which ranked at only eighth among the ten extracts (Table 1). Sc contained relatively low polyphenol content (3.6 mg) but showed the strongest antioxidative abil-

Table 1. Medical herbs selected for this study

Korean name	Scientific name	Origin	Total amounts of extracts (mg/ml)	Total mounts of polyphenols (mg/g of extract)	Antioxidative activity (% at 5 mg/ml)
Sanyak	*Dioscorea batats* (Db)	Korea	14.7	2.48±0.22	32.3±1.1
Baekchul	*Atractylodes macrocephala* (Am)	China	18.9	3.79±0.19	49.4±0.1
Gamcho	*Glycyrrhiza uralensis* (Gu)	China	39.6	12.34±1.01	70.3±2.0
Hwanggi	*Astragalus membranaceus* (Ame)	Korea	18.9	2.41±0.11	27.6±1.5
Magamog	*Sorbus commixta* (Sc)	Korea	5.5	3.57±0.14	89.2±0.1
Saenggang	*Zingiber officinale* (Zo)	Korea	2.7	1.44±0.05	70.5±0.2
Omija	*Schisandra chinensis* (Sch)	Korea	51.4	8.31±0.27	51.5±2.1
Sansuyu	*Cornus officinalis* (Co)	Korea	62.8	15.73±1.40	87.8±1.1
Gugija	*Lycium chinense* (Lc)	Korea	52.3	21.57±0.31	33.9±6.6
Danggui	*Angelica gigas* (Ag)	Korea	54.3	8.51±0.43	44.0±1.6

ity, yielding 89.2% of radical scavenging ability at the same concentration (Table 1). Co extract included high level of polyphenols and also demonstrated potent antioxidative ability (Table 1).

In vitro cytotoxicity test of herbal extracts

To examine the cytotoxicity of the extracts, MA104 and Vero cells were treated with various concentrations of the extracts for 24 hr at 37℃, and cell viability was measured by MTT assay. The extracts of Lc, Am, Db, and Ame exhibited no cytotoxicity on either cell lines at all concentrations tested (Fig. 1). Gu extract showed cytotoxicity at 250~1,000 μg/ml on MA104 cells and at 500~1,000 μg/ml on Vero cells. Ag extract showed cytotoxicity at 500~1,000 μg/ml on MA104 cells and 250~1,000 μg/ml on Vero cells (Fig. 1). Sc extract exhibited cytotoxicity at 125~1,000 μg/ml on MA104 cells and only at 1,000 μg/ml on Vero cells, displaying different cytotoxicity profiles between the two cell lines (Fig. 1). Co extract was not cytotoxic to Vero cells but showed slight cytotoxicity at 1,000 μg/ml on MA104 cells (Fig. 1). Zo extract showed cytotoxicity at 500~1,000 μg/ml on both cell lines (Fig. 1). Sch extract was cytotoxic to both cell lines at 1,000 μg/ml (Fig. 1). In summary, six of ten extracts displayed cytotoxicity at high concentrations of 125~1,000 μg/ml, and four extracts showed no cytotoxicity up to 1,000 μg/ml. Additionally, cytotoxicity of the extracts were also measured in MDCK cells (data not shown). Cytotoxic concentrations of the extract on three cell lines are summarized in Table 2. According to cytotoxic concentration of each extract, Lc extract was shown to be the safest. Ame extract is the second and Db and Am extracts were the third safe (Table 2).

Table 2. Cytotoxic concentrations of herbal extracts on three cell lines

Herbal extracts	Cytotoxic concentration (μg/ml)		
	MA104	Vero	MDCK
Sc	125	1,000	250
Co	1,000	> 1,000	1,000
Zo	250	250	125
Gu	250	250	500
Sch	> 1,000	1,000	500
Am	> 1,000	> 1,000	500
Ag	500	250	500
Lc	> 1,000	> 1,000	> 1,000
Db	> 1,000	> 1,000	500
Ame	> 1,000	> 1,000	1,000

Virucidal activity of herbal extracts against HAV and RV

HAV is a non-enveloped virus and contains a single-stranded RNA genome. HAV is a contagious virus that can cause liver disease in humans and there is no specific treatment for HAV infections. To examine the virucidal activity of the extracts, the virus was incubated with the extracts, and the residual viral infectivity was analyzed. Since HAV does not form plaques in cell cultures, viral infectivity was analyzed by quantification of viral gene copies in the infected cells. Except for Gu extract, nine extracts showed variable levels of virucidal activities against HAV (Fig. 2A). Gu extract rarely reduced the viral infectivity at the concentration of 100 μg/ml, permitting the similar viral gene copies the control (Fig. 2A). In contrast, nine extracts reduced the viral infectivity at concentrations that did not show cytotoxicity. Sc extract reduced the viral infectivity by greater than 90% and 99% at concentration of 10 μg/ml and 100 μg/ml, respectively, displaying the most robust virucidal activity

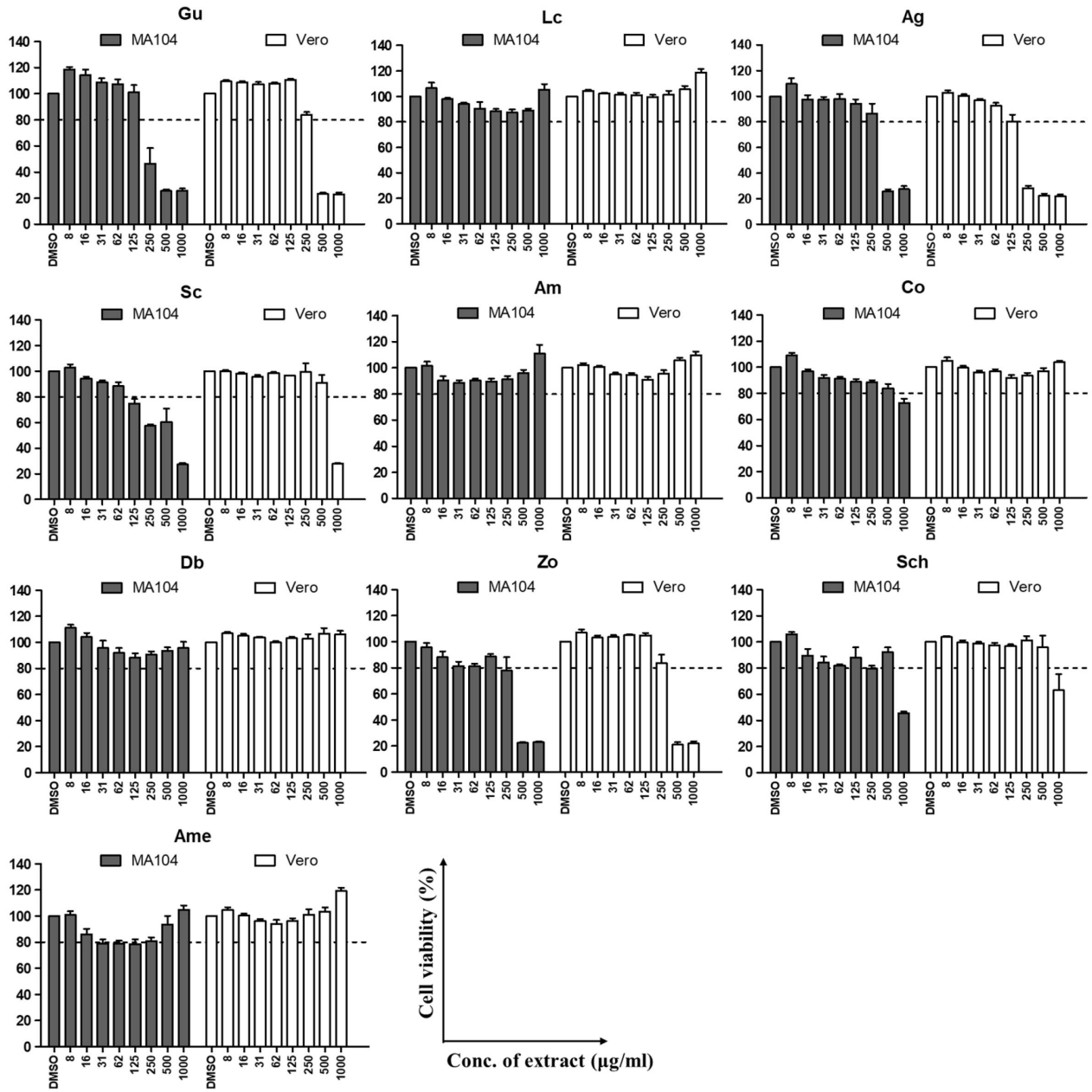

Fig. 1. *In vitro* cytotoxicity of herbal extracts. Ethanol extracts were treated into MA104 and Vero cells at various concentrations of 8~1,000 μg/ml for 24 hr at 37℃, with DMSO used as the vehicle control. Cell viability was measured by MTT assay, and the concentration of extract that showed cell viability less than 80% was considered as a cytotoxic concentration.

among the extracts (Fig. 2A). Co extract also efficiently inactivated the viruses by greater than 90% and 99% at concentration of 50 μg/ml and 500 μg/ml, respectively (Fig. 2A). In addition, the extracts of Lc, Am, and Db decreased the viral infectivity greater than 90% at concentration of 1,000 μg/ml, 1,000 μg/ml, and 500 μg/ml, respectively (Fig. 2A). The extracts of Sch, Ame, Ag, and Zo showed partial but significantly apparent virucidal activities at non-cytotoxic concentrations (Fig. 2A).

RV is a non-enveloped virus possessing a segmented RNA genome. RV is the most common cause of diarrheal disease among infants and young children, and there is no specific treatment for RV infections. We also evaluated the virucidal effects of the extracts against RV. After incubation of the extract and RV, the mixture was added to Vero cells for viral infection. Among the extracts, Gu, Ag, Sc, Co, and Ame extracts resulted in significant increases in cell viability as compared to the virus control (PC), suggesting that the extracts

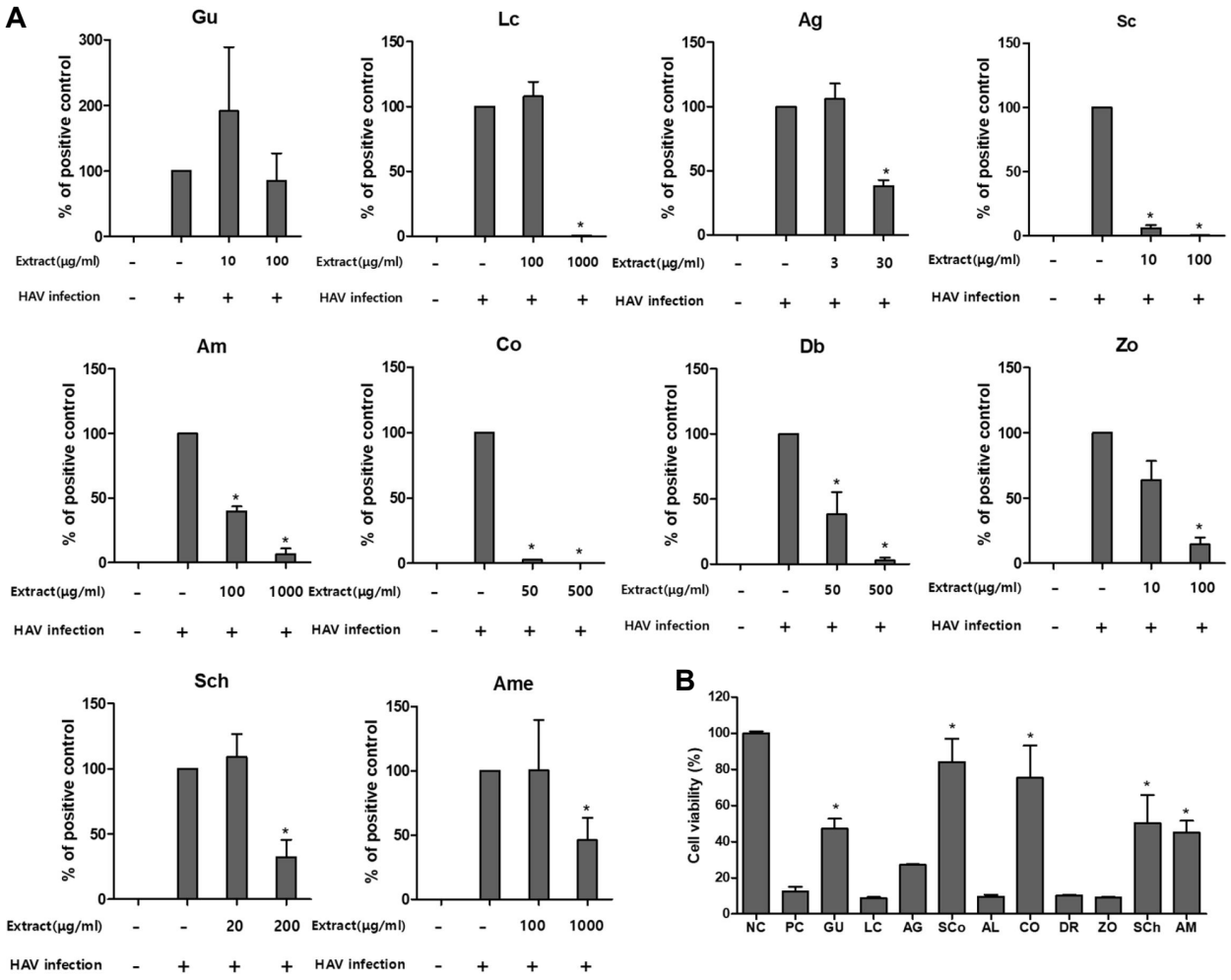

Fig. 2. Virucidal activity of herbal extracts against HAV and RV. (A) Virucidal activity of extracts against HAV. HAV was mixed with each of the extract at non-cytotoxic concentrations for 24 hr at 37℃. The mixtures were then added to Vero cells for viral infection for three days. Relative HAV RNA copies to the virus infection control were shown. (B) Virucidal activity of extracts against RV. Ten MOI of RV was mixed with 100 μg/ml of the extract and incubated for 24 hr at 37℃. The mixtures were added to Vero cells for viral infection. Three days later, cell viability was measured by MTT assay. The cell viability of extract-treated samples was compared to virus infection control (PC) for statistical significance.

inactivated the viruses (Fig. 2B). These results together demonstrate that the extracts of medical herbs possess potent virucidal activity against HAV and RV.

Herbal extracts as inactivating agents for the preparation of inactivated influenza vaccine

Considering the potent virucidal effects of the extracts, we investigated whether the extracts can be used as novel inactivating agents for preparation of an inactivated viral vaccine. Based on virucidal effects and cytotoxicity results, five extracts of Lc, Sc, Zo, Co, Gu were tested for immune-enhancing effect. RAW264.7 cells, murine macrophage cell line, was co-treated with the five extracts with LPS, and secreted proinflammatory cytokines were measured. None of the five extracts increased TNF-α production, as compared to LPS (Fig. 3A). However, the extracts of Lc and Co significantly increased the production of IL-1β, as compared to LPS (Fig. 3A). In addition, Zo extract increased the production of IL-6 as compared to LPS (Fig. 3A). NO is a ubiquitous cellular signaling molecule, synthesized in a variety of cells including macrophages. It has been reported that NO has a versatile functions in innate immunity and inflammation [2]. While none of the extracts increased NO synthesis, the extracts of Zo, Co, and Gu significantly decreased LPS-induced synthesis of NO (Fig. 3A).

Next, the virucidal effects of the five extracts were exam-

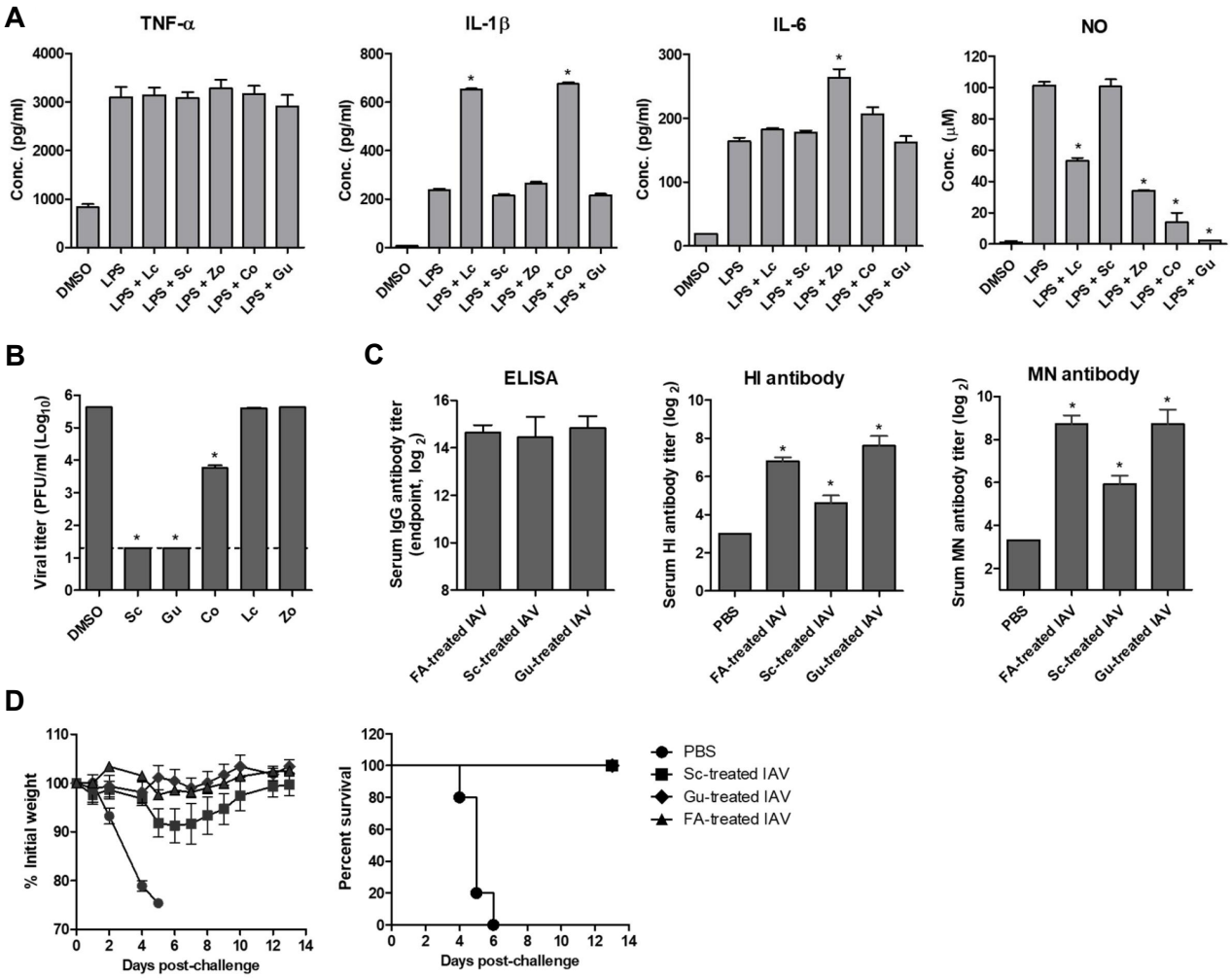

Fig. 3. Herbal extracts as inactivating agents for preparation of an inactivated influenza vaccine. (A) Effects of extracts on proinflammatory cytokines production and NO synthesis in RAW264.7 cells. Each of five extracts and LPS were co-treated to RAW264.7 cells and cytokine production and NO synthesis was measured and compared to those of LPS-treated cells. (B) Virucidal activity of five extracts against IAV. 5×10^5 PFUs of A/PR/8/34 virus was mixed with 100 μg/ml of the extract for 24 hr at 37℃. The mixtures were subjected to plaque assay for viral titration. (C) Induction of antibodies after vaccination with extract-treated IAV in a mouse model. Groups of mice (n=6) were vaccinated with two doses of extract-treated IAV with the interval of two weeks. Serum ELISA antibodies, HI antibodies, and MN antibodies were measured and compared to those of FA-treated IAV. (D) Protective efficacy of extract-treated IAV against lethal challenge. Weight changes (left) and survival rates (right) of the vaccinated mice after the lethal challenge were monitored daily (n=6).

ined against IAV. When the extracts were incubated with IAV, the extracts of Sc and Gu completely inactivated the viruses, while Co extract partially inactivated the viruses (Fig. 3B). The extracts of Lc and Zo did not inactivate the viruses (Fig. 3B). To examine the immunogenicity of inactivated IAV *in-vivo* animal model, Sc extract- and Gu extract-treated IAV was inoculated into mice, and vaccine-induced antibody responses were analyzed, with formalin (FA)-treated IAV used as a control. In ELISA, Sc extract- and Gu-treated IAV elicited high levels of antibodies, similar to that of FA-treated IAV (Fig. 3C). Of note, Gu extract-treated IAV induced the similar or slightly higher levels of neutralizing antibodies than FA-treated IAV (Fig. 3C). The Sc extract-treated IAV induced lower levels of neutralizing antibodies than FA-treated IAV. To evaluate the protective efficacy of the inactivated vaccines, vaccinated mice were challenged with lethal dose of wild type virus. Upon the challenge, non-vaccinated mice group (PBS) rapidly lost their weights and all died within a week (Fig. 3D). In contrast, vaccination with Gu extract-treated IAV, similar to FA-treated IAV, effectively protected the mice against lethal infection, without causing weight loss and death (Fig. 3D). Mice vaccinated with Sc

extract-treated IAV developed mild weight loss of approximately 10% but all survived the lethal infection (Fig. 3D). The results suggest that the extracts of Sc and Gu can be used as alternative inactivating agents for the preparation of inactivated influenza vaccines.

Discussion

The development of antiviral agents and vaccine platforms are considered as critical for preparedness against newly emerging infectious diseases. It has been well recognized that natural products serve as a resource for the discovery of antiviral drugs. Many studies have shown that natural products show broad-spectrum antiviral activity against various unrelated viruses through different mechanisms. For instance, green tea catechins have been shown to exert extremely broad-spectrum antiviral activity against unrelated different viruses, including HIV, HSV, EBV, HBV, HCV, and influenza virus [28]. It was also shown that green tea catechins exhibited virucidal activity against influenza virus and dengue virus [20]. In the study, covalent cross-linking between natural compounds and viral surface proteins was suggested as a major explanation for the virucidal ability [20].

There is currently no specific treatment for HAV and RV, and only one antiviral drug, oseltamivir, is approved for the treatment of IAV. Many studies have reported the antiviral activities of plant-derived natural products against HAV [13, 18, 19]. In addition, various natural products have been screened to possess antiviral activities against RV [9, 15]. Those studies together suggest that plant extracts provide promising starting materials for identifying novel antiviral agents, with our results further expanding the resource. In this study, we examined the virucidal effects of herbal extracts against HAV, RV, and IAV. With varying degree of efficacy, nine from ten extracts displayed virucidal activities against HAV (Fig. 2A), and five extracts of Gu, Sc, Co, Sch, Ame inactivated the RV (Fig. 2B). In addition, three extracts of Sc and Gu completely removed the viral infectivity of IAV (Fig. 3B). The results suggest that herbal extracts present a vast and promising pool from which novel antiviral drugs can be discovered. Consistent with previous observations, the extracts tested in this study demonstrated broad-spectrum virucidal activity against multiple unrelated viruses, including HAV, RV, and IAV. Given that HAV and RV are non-enveloped viruses distinct from IAV that is enveloped virus, virucidal effects of the extract encompass enveloped and non-enveloped viruses. Further studies are needed to determine individual compounds responsible for virucidal activity and their molecular mechanisms for the development of clinically relevant antiviral drugs. In addition, it would be worthwhile to analyze the non-virucidal antiviral activity of the extracts in virus-infected cells by determining the viral or host targets of individual compounds contained in the extracts.

Currently approved inactivated viral vaccines are prepared by viral inactivation with toxic, carcinogenic chemicals such as FA or β-propiolactone [12]. Therefore, additional purification steps are required to remove the toxic reagents. Moreover, it was previously reported that FA-inactivated vaccines occasionally led to the phenomenon termed antibody-dependent enhancement of viral infection, in which vaccination resulted in increased disease severity upon infection, rather than protecting it [11, 26]. Thus, development of inactivating agents based on plant-derived natural compounds provides novel strategies to design a safe and effective inactivated vaccine platform.

Our results demonstrate the wide potentials of herbal extracts as antiviral agents as well as inactivating agents. Although the present study described the virucidal effects of extracts containing a number of single compounds, our results provide a reasonable basis for further studies to discover novel natural compounds possessing prophylactic and therapeutic potentials.

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 한약재 식물 에탄올추출물을 이용한 인플루엔자 불활화백신 제작

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코로나-19 펜데믹에서 볼 수 있듯이, 새로운 바이러스 감염병의 출현은 전 세계적으로 공중보건에 심각한 우려를 발생시킨다. 특히, 항바이러스제 및 백신의 부재는 감염병의 피해를 더욱 증가시킨다. 식물 유래 천연물은 안전하고 효과적인 항바이러스제 개발의 주요 공급원이다. 본 연구는 한약재 식물의 에탄올추출물의 항바이러스 활성을 분석함으로써 안전성과 효능을 갖는 새로운 항바이러스제 후보물질을 발굴하는 것을 목표로 하였다. 10종의 한약재 에탄올추출물의 항산화활성과 세포독성을 분석한 후 로타바이러스, A형간염바이러스, 독감바이러스에 대한 광범위한 바이러스 사멸활성을 분석하였다. 특히, 마가목과 감초의 추출물은 독감바이러스에 대한 강력한 사멸활성을 나타내었다. 또한, 마가목과 감초의 추출물로 사멸된 독감바이러스의 백신효능과 방어효능을 마우스 모델에서 검증하였다. 추출물로 사멸된 바이러스는 높은 수준의 중화항체를 유도하였으며 야생형 바이러스 공격접종을 효과적으로 방어하는 우수한 백신효능을 나타내었다. 본 연구의 결과는 한약재 유래 천연물을 기반으로 하는 항바이러스제와 사백신 제조를 위한 바이러스 불활화제 개발에 활용될 수 있는 가능성을 제시한다.