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Research article

In vitro and *in vivo* anti-inflammatory activities of Korean Red Ginseng-derived components

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ABSTRACT

Background: Although Korean Red Ginseng (KRG) has been traditionally used for a long time, its anti-inflammatory role and underlying molecular and cellular mechanisms have been poorly understood. In this study, the anti-inflammatory roles of KRG-derived components, namely, water extract (KRG-WE), saponin fraction (KRG-SF), and nonsaponin fraction (KRG-NSF), were investigated.
Methods: To check saponin levels in the test fractions, KRG-WE, KRG-NSF, and KRG-SF were analyzed using high-performance liquid chromatography. The anti-inflammatory roles and underlying cellular and molecular mechanisms of these components were investigated using a macrophage-like cell line (RAW264.7 cells) and an acute gastritis model in mice.
Results: Of the tested fractions, KGR-SF (but not KRG-NSF and KRG-WE) markedly inhibited the viability of RAW264.7 cells, and splenocytes at more than 500 μg/mL significantly suppressed NO production at 100 μg/mL, diminished mRNA expression of inflammatory genes such as inducible nitric oxide synthase, cyclooxygenase-2, tumor necrosis factor-α, and interferon-β at 200 μg/mL, and completely blocked phagocytic uptake by RAW264.7 cells. All three fractions suppressed luciferase activity triggered by interferon regulatory factor 3 (IRF3), but not that triggered by activator protein-1 and nuclear factor-kappa B. Phospho-IRF3 and phospho-TBK1 were simultaneously decreased in KRG-SF. Interestingly, all these fractions, when orally administered, clearly ameliorated the symptoms of gastric ulcer in HCl/ethanol-induced gastritis mice.
Conclusion: These results suggest that KRG-WE, KRG-NSF, and KRG-SF might have anti-inflammatory properties, mostly because of the suppression of the IRF3 pathway.

1. Introduction

Inflammation is an innate immune response consisting of a series of complex biological processes to protect the body from infection by pathogens, including bacteria, viruses, and fungi [1,2]. Inflammation is characterized by key symptoms, including recruitment of white blood cells, pain, redness, swelling, heat, tissue damage, and organ dysfunction. During the inflammatory response, different types of immune cells are actively recruited to the inflamed lesions to remove the invading pathogens. Among these immune cells, the macrophage is one of the major effector cells governing inflammatory responses by producing various inflammatory mediators, including nitric oxide (NO), reactive oxygen/nitrogen species (ROS/RNS), prostaglandin E_2 (PGE_2), and different types of proinflammatory cytokines. The latter include tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 [under the control of the activator protein (AP)-1], nuclear factor kappa B (NF-κB), and interferon (IFN) regulatory factor 3 (IRF3) [2–7]. Although inflammation is a host defense mechanism to protect the body from invading pathogens, chronic inflammation, which is a prolonged

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state affecting tissue remodeling for several weeks to years, is regarded as a leading cause in the development of a variety of diseases, such as inflammatory/autoimmune diseases, neurodegenerative diseases, and cancers [8–10].

Korean ginseng (*Panax ginseng*) is a perennial plant that has been traditionally used as an herbal medicine to ameliorate the symptoms of various diseases in eastern Asia. The ginseng root has been used as a common ethnopharmacological remedy to support vitality [11,12]. Because fresh ginseng is easily degraded at room temperature, it needs to be processed to red ginseng by steaming and drying, and accumulating evidence has revealed that red ginseng has higher biological activity and lower side effects compared to fresh or white ginseng [13]. Korean Red Ginseng (KRG) has been known to have various biological activities, including immune enhancement, antioxidant effects, memory enhancement, improvement of menopausal disorder, and induction of metabolic energy [14–17]. However, although KRG has been studied in human health and immunity, the therapeutic potential of each component derived from KRG extract in modulating inflammatory responses and in preventing inflammatory diseases has been poorly understood.

Therefore, in this study, we prepared three fractions of KRG—water extract (KRG-WE), nonsaponin fraction (KRG-NSF), and saponin fraction (KRG-SF)—and investigated their therapeutic potency in inflammatory responses and diseases using lipopolysaccharide (LPS)-stimulated macrophages and an acute inflammatory gastritis mouse model.

2. Materials and methods

2.1. Materials

KRG-WE, KRG-NSF, and KRG-SF were kindly supplied by the Korea Ginseng Corporation (Daejeon, Korea). Male imprinting control region (ICR) mice (6–8 wk old, 17–21 g) were purchased from Orient Bio (Gyeonggi, Korea). RAW264.7 and HEK293 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Dulbecco's modified Eagle's medium, Roswell Park Memorial Institute 1640 (RPMI 1640) medium, fetal bovine serum (FBS), streptomycin, penicillin, and L-glutamine were purchased from Gibco (Grand Island, NY, USA). LPS, N^{ω}-nitro-L-arginine methyl ester (L-NAME), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), polyethylenimine (PEI), ranitidine (RNT), and fluorescein isothiocyanate (FITC)-dextran were purchased from Sigma (St. Louis, MO, USA). The primers used for semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) were synthesized, and PCR premix was purchased from Bioneer Inc. (Daejeon, Korea). Constructs expressing signaling proteins (FLAG-MyD88 and FLAG-TBK1) and luciferase constructs containing the binding promoters for NF-κB, AP-1, and IFN-β were used as previously reported [18,19]. Antibodies used in this study were purchased from Cell Signaling Technology (Beverly, MA, USA). The luciferase assay system was purchased from Promega (Madison, WI, USA).

2.2. Mice

Male ICR mice (6–8 wk old, 17–21 g) were obtained from Orient Bio (Gyeonggi, Korea) and maintained in plastic cages under standard conditions. Water and pelleted food (Samyang, Daejeon, Korea) were supplied *ad libitum*. Studies were performed in accordance with the guidelines established by the Institutional Animal Care and Use Committee at Sungkyunkwan University, Suwon, Korea.

Table 1
Primer sequences used for semiquantitative PCR

Name		Sequence (5′ to 3′)
iNOS	F	CCCTTCCGAAGTTTCTGGCAGCAG
	R	GGCTGTCAGAGCCTCGTGGCTTTGG
COX-2	F	CACTACATCCTGACCCACTT
	R	ATGCTCCTGCTTGAGTATGT
TNF-α	F	TTGACCTCAGCGCTGAGTTG
	R	CCTGTAGCCCACGTCGTAGC
IFN-β	F	CAGGATGAGGACATGAGCACC
	R	CTCTGCAGACTCAAACTCCAC
GAPDH	F	CACTCACGGCAAATTCAACGGCA
	R	GACTCCACGACATACTCAGCAC

COX-2, cyclooxygenase-2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IFN-β, interferon beta; iNOS, inducible nitric oxide synthase; PCR, polymerase chain reaction; TNF-α, tumor necrosis factor alpha

2.3. Preparation of splenocytes from mice

Splenocytes from ICR mice were prepared as previously described [20].

2.4. Cell culture

RAW264.7, HEK293 cells and splenocytes were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, glutamine, and antibiotics (penicillin and streptomycin) at 37°C in a 5% CO_2 humidified incubator.

2.5. High-performance liquid chromatography analysis

For determination of ginsenosides from KRG-WE, KRG-SF, and KRG-NSF, high-performance liquid chromatography (HPLC) was conducted as described previously [21,22].

2.6. Cell viability assay

The cytotoxic effects of KRG-WE, KRG-SF, and KRG-NSF were determined using the MTT assay, as reported previously [23].

2.7. Phagocytosis assay

RAW264.7 cells treated with KRG-WE, KRG-SF, or KRG-NSF were resuspended in 100 μL phosphate-buffered saline (PBS) containing 1% human AB serum and incubated with FITC-dextran (1 mg/mL) at 37°C for 30 min. The incubations were stopped by adding 2 mL ice-cold PBS containing 1% human serum and 0.02% sodium azide. The cells were then washed three times with cold PBS-azide and analyzed on a FACScan flow cytometer, as reported previously [24].

2.8. NO production assay

RAW264.7 cells were pretreated with either KRG-WE, KRG-NSF, or KRG-SF and incubated with LPS (1 μg/mL) for 24 h. NO production level was determined using Griess reagent as described previously [25].

2.9. mRNA analysis by semiquantitative RT-PCR

RAW264.7 cells pretreated with KRG-WE, KRG-NSF, or KRG-SF for 1 h were incubated with LPS (1 μg/mL) for 6 h. Total RNA was isolated with TRI reagent according to the manufacturer's instructions and stored at −70°C until use. cDNA from 1 μg of total RNA was synthesized using MuLV reverse transcriptase according to the manufacturer's instructions, and semiquantitative RT-PCR