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A Formulated Korean Red Ginseng Extract Inhibited Nitric Oxide Production through Akt- and Mitogen Activated Protein Kinase-dependent Heme Oxygenase-1 Upregulation in Lipoteichoic Acid-stimulated Microglial Cells

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Korean red ginseng made from steaming and drying fresh ginseng has long been used as a traditional herbal medicine due to its effects on the immune, endocrine, and central nerve systems and its anti-inflammatory activity. In this study, we investigated the molecular mechanism responsible for the anti-inflammatory effects of a formulated Korean red ginseng extract (RGE) in response to lipoteichoic acid (LTA), a cell wall component of gram-positive bacteria. RGE inhibited LTA-induced nitric oxide (NO) secretion and inducible nitric oxide synthase (iNOS) expression in BV-2 microglial cells, without affecting cell viability. RGE also inhibited nuclear translocation of nuclear factor kappa B (NF-κB) p65 and degradation of IκB-α. In addition, RGE increased the expression of heme oxygenase-1 (HO-1) in a dose-dependent manner, and the inhibitory effect of RGE on iNOS expression was abrogated by small interfering RNA-mediated knockdown of HO-1. Moreover, RGE induced nuclear translocation of nuclear factor E2-related factor 2 (Nrf2), a transcription factor that regulates HO-1 expression. Furthermore, the phosphoinositide-3-kinase (PI-3K) inhibitor and mitogen-activated protein kinase (MAPK) inhibitors suppressed RGE-mediated expression of HO-1, and RGE enhanced the phosphorylation of Akt, extracellular signal-regulated kinases (ERKs), p38, and c-JUN N-terminal kinases (JNKs). These results suggested that RGE suppressed the production of NO, a proinflammatory mediator, by inducing HO-1 expression via PI-3K/Akt- and MAPK-dependent signaling in LTA-stimulated microglia. The findings indicate that RGE could be used for the treatment of neuroinflammation induced by gram-positive bacteria and that it may have therapeutic potential for various neuroinflammation-associated disorders.

Key words : Heme oxygenase-1, Korean red ginseng, neuroinflammation, nitric oxide, Nrf2

Introduction

Gram-positive bacterial infections of the central nervous system (CNS) cause bacterial meningitis, encephalomyelitis, brain abscess or sepsis [26]. Bacterial invasion induces a rapid inflammatory response, which is mediated by the brain's innate immune cells such as microglia. Lipoteichoic acid (LTA) is a cell wall component of Gram-positive bacteria such as *Staphylococcus aureus* [13]. Several studies have demonstrated that LTA may bind to target cells through Toll-like receptors-2 (TLR-2) which plays a critical role in LTA-induced microglial activation [31]. Signaling via TLR-2 is mediated by different adaptor proteins, including MyD88, which promotes downstream signaling via mitogen-activated protein kinases (MAPKs), and nuclear factor-kappaB (NF-κB) leading to the expression of pro-inflammatory molecules [23]. In Gram-positive infection, microglia produce considerable amounts of nitric oxide (NO) through expression of inducible nitric oxide synthase (iNOS). Large amounts of NO function to restore CNS homeostasis by clearing pathogens and infected cells. However, deregulated or chronic activation of microglial cells can induce too many pro-inflammatory molecules including NO, leading to neuronal cell death and brain injury [24]. Moreover, the neuroinflammatory responses of the CNS are well-known features of various neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease. Therefore control of microglial activation and subsequent suppression of the production of neurotoxic pro-inflammatory molecules could provide a potential therapeutic approach for the treatment of neurodegenerative diseases as well as meningitis [38].

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Heme oxygenase-1 (HO-1) is an inducible enzyme that catalyzes the oxidation of cellular heme into carbon monoxide (CO), biliverdin, and free iron [30]. HO-1 and its enzymatic by-products provide a host defense mechanism that can protect the body against oxidative injury and also contributes to the anti-inflammatory activity of cells and tissues [30]. In activated macrophages, HO-1 expression or CO treatment inhibits the production of the pro-inflammatory mediators such as NO, prostaglandin E2 (PGE2), and inflammatory cytokines [28]. Moreover, a large number of remedial agents have been reported to induce HO-1 expression and exert their anti-inflammatory effects through HO-1 induction.

The root of Korean *Panax ginseng* C.A. Meyer has been used as a traditional herbal medicine not only in Korea but also in Asia and Western countries. It has been reported that ginseng and ginseng saponins have a wide range of pharmacological activities including anti-tumorigenic, immune-stimulating, antistress and antioxidant effects [3, 5, 7, 9, 33, 39]. It is especially well established that ginseng ameliorates inflammatory responses [14, 29, 41]. Red ginseng is made by steaming and drying the fresh ginseng. The pharmacological efficacy of Korean red ginseng is known to be enhanced by these processes mostly due to the changes in the characteristics of the constituent ginsenosides [11]. In the present study, we investigated the molecular mechanism responsible for the anti-inflammatory effects of a formulated Korean red ginseng extract (RGE) in LTA-stimulated microglia. We elucidated that RGE inhibits LTA-induced iNOS expression via upregulation of HO-1.

Materials and Methods

Materials

Lipoteichoic acid from *Staphylococcus aureus*, and other reagents not referred were purchased from Sigma (St. Louis, MO, USA). A formulated Korean red ginseng extract (Hansamin Gold) was purchased from Nonghyup Red Ginseng (Seoul, Korea). HO-1 siRNA, and antibodies for iNOS, HO-1, NF-κB p65, inhibitor of kappa B-alpha (IkB-a), histone deacetylase 3 (HDAC3), ERK, JNK, p38, Akt, α-tubulin, and b-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibody against for phosphorylated ERK (p-ERK), p-JNK, p-p38, p-Akt were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibody against for TATA-binding protein (TBP) was purchased from Cloudclone corp (Katy, Texas, USA). Cell culture medium, DMEM, and fetal bovine serum (FBS) were purchased from Gibco BRL (now part of Invitrogen Corporation, Carlsbad, CA, USA). X-treme GENE siRNA Transfection Reagent were purchased from Roche (Indianapolis, IN, USA).

Cell culture

Mouse BV-2 microglial cells were grown in DMEM medium supplemented with 5% heat inactivated fetal bovine serum (FBS) and 0.1% penicillin-streptomycin (BioSource International, Camarillo, CA, USA) at 37℃ in a humidified atmosphere of 5% CO_2 and 95% air.

Cell viability assay (MTT assay)

The cytotoxicity of RG was assessed using the microculture tetrazolium (MTT)-based colorimetric assay. The remaining cells after Griess reaction were used for MTT assay. MTT was added to each well (final concentration is 62.5 μg/ml). After incubation for 3 hr at 37℃ in 5% CO_2, the supernatant was removed and the formazan crystals produced in viable cells were solubilized with dimethylsulfoxide (DMSO). The absorbance of each well was then read at 570 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

Measurement of nitrite concentration

NO synthesis in cell cultures was measured by a microplate assay method. To measure nitrite, 100 ml aliquots were removed from conditioned medium and incubated with an equal volume of the Griess reagent (1% sulfanilamide/0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride/2.5% H3PO4) at room temperature for 10 min. Nitrite concentration was determined by measuring the absorbance at 540 nm with a microplate spectrophotometer (Bio-Rad, Hercules, CA, USA). The sodium nitrite was used as a standard.

Transient transfection with siRNA

Transfection of cells with siRNA was performed using the X-treme GENE siRNA Transfection Reagent (Roche Applied Science), according to the manufacturer's instructions. Commercially available human HO-1 and Nrf-2 specific siRNAs (Santa Cruz, Heidelberg, Germany) and negative control siRNAs (Santa Cruz) were used for transfection. In brief, X-treme GENE siRNA Transfection Reagent (10 μl) was added to 100 μl serum-free medium containing 2 μg of each siRNA oligo, and was incubated for 20 min at room temperature. Gene silencing was measured after 48 hr by Western blotting.

Preparation of nuclear extracts

Nuclear extracts were prepared as described previously [2]. Cells were washed with PBS, resuspended in ice-cold isotonic buffer A [10 mM HEPES (pH7.9) 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol (DTT) and protease inhibitor cocktail], and incubated at 4℃ for 10 min. Cells were centrifuged at 15,000× g for 1 min and pellet was resuspended in ice-cold buffer B [20 mM HEPES (pH 7.9), 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 25% glycerol, 0.5 mM DTT and protease inhibitor cocktail] followed by incubation at 4℃ for 30 min with occasional vortexing. The resulting suspension was centrifuged at 15,000x g for 5 min, and the supernatant was stored at -20℃.

Western blot analysis

Cells were harvested in ice-cold lysis buffer (1% Triton X-100 and 1% deoxycholate in PBS). The protein content of the cell lysates was determined using Bradford reagent (Bio-Rad; Hercules, CA, USA). The proteins in each sample were resolved by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to a polyvinylidene difluoride (PVDF) membrane, and incubated with the appropriate antibodies. The proteins were visualized using an enhanced chemiluminescence detection system (Amersham Biosciences, Piscataway, NJ, USA) with horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies. Anti-a-tubulin or anti-b-actin antibodies were used as loading control for cytosolic protein and anti-HDAC3 or anti-TBP antibodies were used as loading control for nuclear protein.

Statistical analysis

All results were expressed as the mean ± SE (standard error). Each experiment was repeated at least three times. Statistical analysis was performed by using SPSS software to determine significant differences. We used one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for comparison of three or more groups. A value of $p<0.05$ was considered statistically significant.

Results

RGE suppresses LTA-inducted neuroinflammatory molecules

To investigate whether RGE could abrogate LTA-mediated neuroinflammation, we examined the effects of RGE on NO production expression of in BV-2 microglial cells. Stimulation of BV-2 microglial cells with LTA increased NO synthesis and iNOS expression, whereas RGE pretreatment significantly attenuated the LTA-induced NO synthesis and iNOS expression in a dose-dependent manner (Fig. 1A, Fig. 1C). To determine the effect of RGE on cell viability, BV-2 microglial cells were treated with various concentration of RGE in the absence or presence of LTA. While LTA induced a little toxicity on BV-2 cells, RGE at concentrations up to

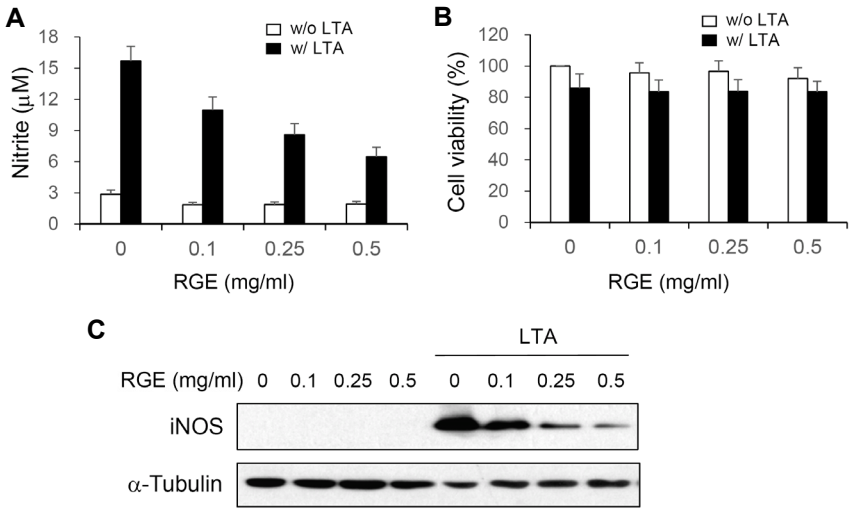

Fig. 1. RGE inhibits NO production and iNOS expression in LTA-stimulated microglial cells. BV-2 cells were treated with different concentrations of RGE for 3 hr and then incubated with or without LTA (10 mg/ml) for 20 hr. Nitrite content was measured using the Griess reaction (A). Cell viabilities were determined by MTT assay (B). Each bar represents the mean ± S.E. from 3 independent experiments. $^{**}p<0.01$ vs the LTA-treated group. The expression of iNOS and α-tubulin were detected by Western blot using specific antibodies (C).

0.5 mg/ml induced no cytotoxicity (Fig. 1B). These results suggest that RGE suppresses NO release in LTA-stimulated microglial cells by inhibiting iNOS expression level, and these effects are not due to cytotoxicity.

RGE suppresses LTA-induced activation of NF-κB

NF-kB is known to mediate the expression of iNOS gene in response to LTA. To determine the effects of RGE on NF-kB activity, we examined nuclear translocation of NF-kB by Western blotting. As shown in Fig. 2, nuclear level of NF-kB p65 was markedly increased and cytosolic level of NF-kB p65 was significantly decreased by LTA treatment. However, RGE pretreatment reduced nuclear level of NF-kB p65 in a dose-dependent manner, at the same time RGE increased cytosolic level of NF-kB p65. In accord with this result, RGE inhibited LTA-induced degradation of IkB-a in a dose-dependent manner. These results suggest that RGE suppresses LTA-induced nuclear translocation of NF-kB via blocking of IkB-a degradation.

RGE reduces iNOS expression through induction of HO-1

To investigate whether RGE induces HO-1 expression in microglia, BV-2 cells were incubated with various concentrations of RGE. The HO-1 protein level was significantly increased after 6 hr by RGE in a dose-dependent manner (Fig. 3A, Fig. 3B). To elucidate that RGE-induced HO-1 suppresses the expression of iNOS, we applied an HO-1 small interfering (si) RNA system to knock down HO-1 function. BV-2 cells were transfected with HO-1 siRNA or control siRNA , and treated with LTA in the absence or presence

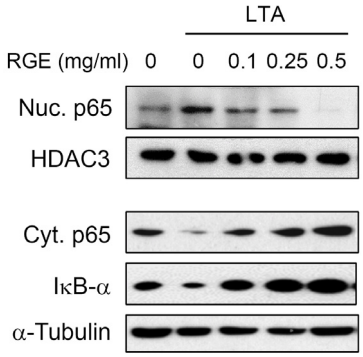

Fig. 2. RGE inhibits LTA-induced NF-κB activation. BV-2 cells were treated with various concentrations of RGE for 3 h and followed by LTA (10 μg/ml) treatment for 1 hr. NF-κB p65 level in nuclear and cytosolic fraction was assessed by Western blotting. The level of IκB-α in cytosolic extracts were also analyzed by Western blotting.

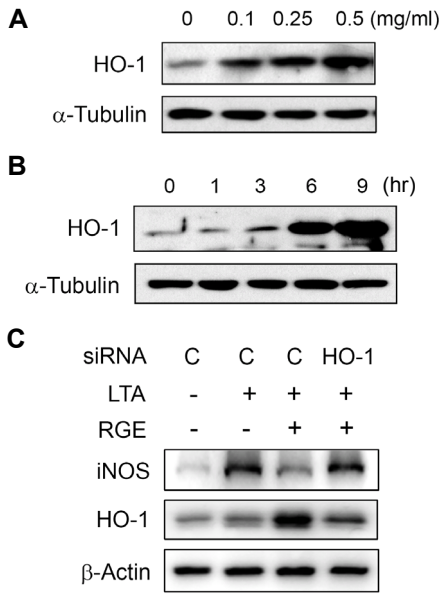

Fig. 3. RGE-induced HO-1 suppresses iNOS expression. BV-2 cells were cultured with increasing concentrations of RGE for 6 hr (A) or with 0.5 mg/ml of RGE for the indicated times (B). HO-1 expression was determined by Western blot. (C) The si-Control or si-HO-1 transfected cells were treated with RGE (0.5 mg/ml) for 3 hr, then stimulated with LTA (10 μg/ml) for 20 hr. The expression iNOS or HO-1 were determined by Western blotting.

of RGE. As shown in Fig. 3C, a decrease of HO-1 blocked RGE-mediated suppression of LTA-stimulated iNOS expression, whereas transfection with control siRNA showed no effect. These results suggest that HO-1 expression is up-regulated by RGE and involved in RGE-induced anti-inflammatory activity.

RGE-induced HO-1 expression is mediated by Nrf2

Since NF-E2-related factor 2 (Nrf2) is known to regulate the expression of HO-1, we investigated whether RGE induces nuclear accumulation of Nrf2, which is critical to its transcriptional activity, in BV-2 cells. As shown in Fig. 4, nuclear level of Nrf2 was increased by RGE in a dose-dependent manner and reached peak at 3 hr. These results suggest that RGE activates Nrf2, which in turn induces HO-1 expression.

PI-3K/Akt and MAPKs mediates RGE-induced HO-1 expression

To elucidate the molecular target of RGE in further upstream signaling pathway of HO-1 expression, we examined the effect of pharmaceutical protein kinase inhibitors of

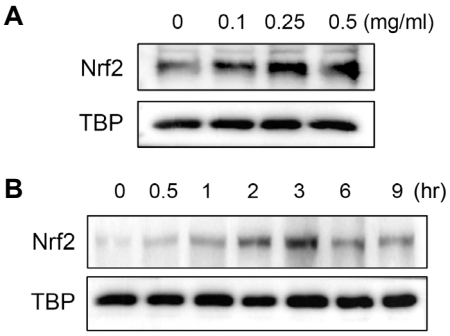

Fig. 4. Effects of RGE on nuclear translocation of Nrf2. BV-2 cells were incubated with indicated concentrations of RGE for 3 hr (A) or with 0.5 mg/ml of RGE for the indicated times (B) and then the levels of Nrf2 in nuclear extracts were analyzed by Western blotting.

MAPKs, PD 98059 (ERK inhibitor), SB203580 (p38 kinase inhibitor), SP600125 (JNK inhibitor), and PI-3K/Akt inhibitor, LY294002. As shown in Fig. 5A, RGE-induced HO-1 expression was significantly inhibited by 3 MAPK inhibitors and slightly inhibited by LY294002. Moreover, RGE induced the phosphorylation of 3 MAPKs and Akt (Fig. 5B). These results indicate that PI-3K/Akt and MAPK signalings occur upstream of RGE-mediated HO-1 expression.

Discussion

In present study, we investigated the anti-inflammatory effects of RGE in LTA-stimulated microglial cells. We used BV-2 microglial cells because there is a close resemblance between BV-2 microglial cells and primary microglia in terms of the inflammatory signaling pathways and BV-2 microglial cells are an appropriate model for the activation of microglia *in vitro*. We found that GRE pretreatment significantly inhibited the production of NO and expression of iNOS in response to LTA without affecting cell viability (Fig. 1). NO is released from activated astrocytes and microglia, and neurons are remarkably sensitive to NO-induced cell death [4, 40]. High amounts of NO produced by iNOS from activated microglial cells are believed to play a critical role in the pathogenesis of various inflammation-related diseases. So suppression of iNOS expression in microglial cells could represent an attractive target to treat various neuroinflammatory diseases [8, 37]. Thus our results suggest that RGE could be a useful remedial agent for Gram-positive bacteria-mediated inflammatory diseases such as pneumonia, meningitis, and sepsis. Red ginseng and several ginsenosides are reported to ameliorate neuroinflammation in response to LPS [15, 16, 19-22, 35]. Our study showed that red ginseng also attenuates LTA-induced neuroinflammatory response. Since LTA is known to provoke inflammatory response through TLR-2 [31], red ginseng might suppress TLR-2 signaling as well as TLR-4.

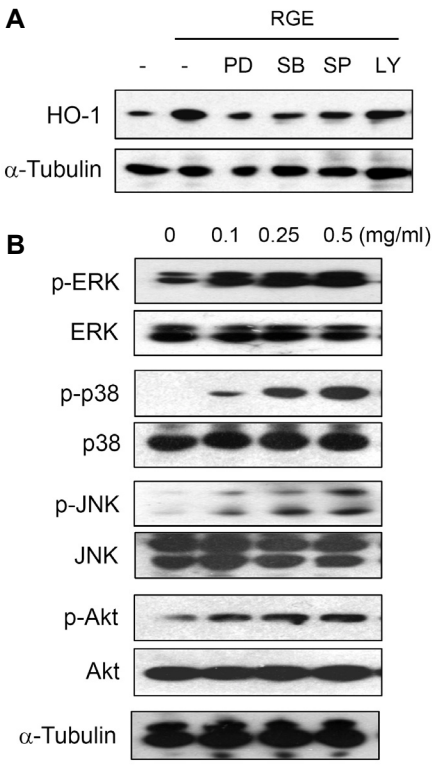

Fig. 5. Involvement of Akt and MAPKs in RGE-mediated expression of HO-1. (A) BV-2 cells were incubated with PD98059 (10 μM), SB203580 (10 μM), SP600125 (10 μM), and LY294002 (10 μM), for 30 min and then treated with RGE (0.5 mg/ml) for 6 hr. (B) Cells were treated with various concentrations of RGE for 30 min. Equal amounts of cytosolic extract were subsequently analyzed by Western blotting with specific antibodies.

A major transcriptional regulator of iNOS genes is NF-κB, which is also a key regulator of a variety of genes involved in immune and inflammatory responses [1]. Inappropriate regulation of NF-κB is directly involved in a wide range of human disorders, including a variety of cancers, neurodegenerative diseases, sepsis, and numerous other inflammatory conditions [6, 10, 32]. Therefore, the development of a drug that controls NF-κB is a promising strategy for the treatment of inflammatory disease [10]. In resting cells, NF-κB dimers remain in the cytosol bound to IkB that block their nuclear import. In response to stimulation, IKK complex phosphorylate IkBs which in turn are rapidly ubiquitinated and degraded by 26S proteasome complex. Consequently,

released NF-κB dimers translocate to the nucleus and stimulate target genes expression [10]. Our study showed that RGE significantly inhibited NF-κB translocation into the nucleus (Fig. 3). In addition, RGE suppressed the LTA-stimulated degradation of IkB-a. Therefore these results suggest that RGE inhibits NF-κB activity through the suppression of degradation of IkB-a.

HO-1 is known to exhibit anti-inflammatory effects by attenuating production of pro-inflammatory mediators [12], and then thought to be used as a potential therapeutic agent for treating inflammatory diseases. We found that RGE significantly increased HO-1 expression in microglial cells (Fig. 3A and B). Since HO-1 expression is reported to be increased by chemical-mediated oxidative stress [17], we elucidated if RGE induced HO-1 expression by cell damage. RGE did not decrease cell viability when we conducted MTT assay. So HO-1 might be specifically induced by RGE, not by cell damage. Furthermore, the blocking of HO-1 expression via siRNA markedly attenuated the inhibitory effects of RGE on LTA-induced iNOS expression (Fig. 3C). These data suggest that RGE inhibit TLR2-mediated iNOS expression via HO-1 induction.

Nrf2 is a transcription factor which plays a central role for inducible expression of HO-1 [34]. In normal conditions, Nrf2 is arrested in the cytoplasm by binding with Kelchlike ECH-associated protein 1 (Keap1) and degraded by the ubiquitin-dependent proteasome. Under activation, Nrf2 is dissociated from Keap1, translocates to the nucleus, and binds antioxidant response elements (AREs) located in the promoter regions of HO-1 [25]. In this study, nuclear translocation of Nrf2 was significantly enhanced by treatment with RGE (Fig. 4). This result suggests that RGE up-regulates HO-1 expression through Nrf2 activation.

Several studies reported that Nrf2 activation and HO-1 expression are regulated by PI-3K and MAPKs such as ERK, JNK, and p38 in response to various stimuli [18, 27]. However, these signaling pathways depend on the type of cells and stimuli in terms of their contribution to HO-1 expression. In this study, RGE-mediated HO-1 expression was suppressed by the inhibitors of PI-3K/Akt, ERK, JNK, and p38 (Fig. 5), and RGE induced the phosphorylation of these kinases. These results suggest that RGE induce HO-1 expression via the activation of PI-3K/Akt, ERK, JNK, and p38. Nrf2 was reported to be phosphorylated at multiple sites by MAPKs to facilitate its nuclear translocation [36]. Therefore, PI-3K/Akt and MAPKs could up-regulate HO-1 through direct phosphorylation of Nrf2 or indirect mechanisms.

In conclusion, we demonstrated that RGE inhibited NO release and iNOS expression in LTA-stimulated microglial cells, and these effects are mediated by PI-3K/Akt and ERK, JNK, p38-dependent HO-1 induction. Our findings suggest that RGE could be used for the treatment of Gram-positive bacteria-induced neuroinflammation and may have therapeutic potential for various neuroinflammation-associated disorders.

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References

1. Ahn, K. S. and Aggarwal, B. B. 2005. Transcription factor NF-κB: A sensor for smoke and stress signals. *Ann. N. Y. Acad. Sci.* **1056**, 218-233.
2. Andrews, N. C. and Faller, D. V. 1991. A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. *Nucleic Acids Res.* **19**, 2499.
3. Attele, A. S., Wu, J. A. and Yuan, C. S. 1999. Ginseng pharmacology: Multiple constituents and multiple actions. *Biochem. Pharmacol.* **58**, 1685-1693.
4. Bal-Price, A. and Brown, G. C. 2001. Inflammatory neurodegeneration mediated by nitric oxide from activated glia-inhibiting neuronal respiration, causing glutamate release and excitotoxicity. *J. Neurosci.* **21**, 6480-6491.
5. Bhattacharya, S. K. and Mitra, S. K. 1991. Anxiolytic activity of *Panax ginseng* roots: An experimental study. *J. Ethnopharmacol.* **34**, 87-92.
6. Blackwell, T. S., Blackwell, T. R., Holden, E. P., Christman, B. W. and Christman, J. W. 1996. In vivo antioxidant treatment suppresses nuclear factor-kappa B activation and neutrophilic lung inflammation. *J. Immunol.* **157**, 1630-1637.
7. Block, K. I. and Mead, M. N. 2003. Immune system effects of echinacea, ginseng, and astragalus: A review. *Integr. Cancer Ther.* **2**, 247-267.
8. Doherty, G. H. 2011. Nitric oxide in neurodegeneration: Potential benefits of non-steroidal anti-inflammatories. *Neurosci. Bull.* **27**, 366-382.
9. Gillis, C. N. 1997. *Panax ginseng* pharmacology: A nitric oxide link? *Biochem. Pharmacol.* **54**, 1-8.
10. Giridharan, S. and Srinivasan, M. 2018. Mechanisms of NF-kappaB p65 and strategies for therapeutic manipulation. *J. Inflamm. Res.* **11**, 407-419.
11. He, M., Huang, X., Liu, S., Guo, C., Xie, Y., Meijer, A. H. and Wang, M. 2018. The difference between white and red

ginseng: Variations in ginsenosides and immunomodulation. *Planta Med.* **84**, 845-854.
12. Jazwa, A. and Cuadrado, A. 2010. Targeting heme oxygenase-1 for neuroprotection and neuroinflammation in neurodegenerative diseases. *Curr. Drug Targets* **11**, 1517-1531.
13. Jiang-Shieh, Y. F., Yeh, K. Y., Wei, I. H., Chang, C. Y., Chien, H. F., Tsai, R. Y., Chang, M. L., Lee, A. W., Pai, M. H. and Wu, C. H. 2005. Responses of microglia *in vitro* to the gram-positive bacterial component, lipoteichoic acid. *J. Neurosci. Res.* **82**, 515-524.
14. Jin, Y., Kotakadi, V. S., Ying, L., Hofseth, A. B., Cui, X., Wood, P. A., Windust, A., Matesic, L. E., Pena, E. A., Chiuzan, C., Singh, N. P., Nagarkatti, M., Nagarkatti, P. S., Wargovich, M. J. and Hofseth, L. J. 2008. American ginseng suppresses inflammation and DNA damage associated with mouse colitis. *Carcinogenesis* **29**, 2351-2359.
15. Kang, A., Hao, H., Zheng, X., Liang, Y., Xie, Y., Xie, T., Dai, C., Zhao, Q., Wu, X., Xie, L. and Wang, G. 2011. Peripheral anti-inflammatory effects explain the ginsenosides paradox between poor brain distribution and anti-depression efficacy. *J. Neuroinflammation* **8**, 100.
16. Kang, A., Xie, T., Zhu, D., Shan, J., Di, L. and Zheng, X. 2017. Suppressive effect of ginsenoside Rg3 against lipopolysaccharide-induced depression-like behavior and neuroinflammation in mice. *J. Agric. Food Chem.* **65**, 6861-6869.
17. Keyse, S. M. and Tyrrell, R. M. 1989. Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite. *Proc. Natl. Acad. Sci. USA.* **86**, 99-103.
18. Kim, J. H., Park, G. Y., Bang, S. Y., Park, S. Y., Bae, S. K. and Kim, Y. 2014. Crocin suppresses LPS-stimulated expression of inducible nitric oxide synthase by upregulation of heme oxygenase-1 via calcium/calmodulin-dependent protein kinase 4. *Mediators Inflamm.* **2014**, 728709.
19. Lee, J. S., Song, J. H., Sohn, N. W. and Shin, J. W. 2013. Inhibitory effects of ginsenoside Rb1 on neuroinflammation following systemic lipopolysaccharide treatment in mice. *Phytother. Res.* **27**, 1270-1276.
20. Lee, K. W., Jung, S. Y., Choi, S. M. and Yang, E. J. 2012. Effects of ginsenoside re on LPS-induced inflammatory mediators in BV2 microglial cells. *BMC Complement. Altern. Med.* **12**, 196.
21. Lee, Y. Y., Park, J. S., Lee, E. J., Lee, S. Y., Kim, D. H., Kang, J. L. and Kim, H. S. 2015. Anti-inflammatory mechanism of ginseng saponin metabolite Rh3 in lipopolysaccharide-stimulated microglia: Critical role of 5′-adenosine monophosphate-activated protein kinase signaling pathway. *J. Agric. Food Chem.* **63**, 3472-3480.
22. Lin, W. M., Zhang, Y. M., Moldzio, R. and Rausch, W. D. 2007. Ginsenoside Rd attenuates neuroinflammation of dopaminergic cells in culture. *J. Neural Transm. Suppl.* **72**, 105-112.
23. Liu, Y., Yin, H., Zhao, M. and Lu, Q. 2014. TLR2 and TLR4 in autoimmune diseases: A comprehensive review. *Clin. Rev. Allergy Immunol.* **47**, 136-147.
24. Lull, M. E. and Block, M. L. 2010. Microglial activation and chronic neurodegeneration. *Neurotherapeutics* **7**, 354-365.
25. Motohashi, H., Katsuoka, F., Engel, J. D. and Yamamoto, M. 2004. Small Maf proteins serve as transcriptional cofactors for keratinocyte differentiation in the Keap1-Nrf2 regulatory pathway. *Proc. Natl. Acad. Sci. USA.* **101**, 6379-6384.
26. Neher, J. J. and Brown, G. C. 2007. Neurodegeneration in models of Gram-positive bacterial infections of the central nervous system. *Biochem. Soc. Trans.* **35**, 1166-1167.
27. Nemmiche, S., Chabane-Sari, D., Kadri, M. and Guiraud, P. 2012. Cadmium-induced apoptosis in the BJAB human B cell line: Involvement of PKC/ERK1/2/JNK signaling pathways in HO-1 expression. *Toxicology* **300**, 103-111.
28. Otterbein, L. E., Bach, F. H., Alam, J., Soares, M., Tao Lu, H., Wysk, M., Davis, R. J., Flavell, R. A. and Choi, A. M. 2000. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat. Med.* **6**, 422-428.
29. Park, J. S., Shin, J. A., Jung, J. S., Hyun, J. W., Van Le, T. K., Kim, D. H., Park, E. M. and Kim, H. S. 2012. Anti-inflammatory mechanism of compound K in activated microglia and its neuroprotective effect on experimental stroke in mice. *J. Pharmacol. Exp. Ther.* **341**, 59-67.
30. Ryter, S. W. and Choi, A. M. 2010. Heme oxygenase-1/carbon monoxide: Novel therapeutic strategies in critical care medicine. *Curr. Drug Targets* **11**, 1485-1494.
31. Schwandner, R., Dziarski, R., Wesche, H., Rothe, M. and Kirschning, C. J. 1999. Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. *J. Biol. Chem.* **274**, 17406-17409.
32. Segain, J. P., Raingeard, de la Bletiere, D., Bourreille, A., Leray, V., Gervois, N., Rosales, C., Ferrier, L., Bonnet, C., Blottiere, H. M. and Galmiche, J. P. 2000. Butyrate inhibits inflammatory responses through NFkappaB inhibition: Implications for crohn's disease. *Gut* **47**, 397-403.
33. Shin, H. R., Kim, J. Y., Yun, T. K., Morgan, G. and Vainio, H. 2000. The cancer-preventive potential of *Panax ginseng*: A review of human and experimental evidence. *Cancer Causes Control* **11**, 565-576.
34. Srisook, K., Kim, C. and Cha, Y. N. 2005. Molecular mechanisms involved in enhancing HO-1 expression: De-repression by heme and activation by Nrf2, the "one-two" punch. *Antioxid. Redox Signal.* **7**, 1674-1687.
35. Sun, X. C., Ren, X. F., Chen, L., Gao, X. Q., Xie, J. X. and Chen, W. F. 2016. Glucocorticoid receptor is involved in the neuroprotective effect of ginsenoside Rg1 against inflammation-induced dopaminergic neuronal degeneration in substantia nigra. *J. Steroid Biochem Mol. Biol.* **155**, 94-103.
36. Sun, Z., Huang, Z. and Zhang, D. D. 2009. Phosphorylation of Nrf2 at multiple sites by MAP kinases has a limited contribution in modulating the Nrf2-dependent antioxidant response. *PLoS One* **4**, e6588.
37. Tieu, K., Ischiropoulos, H. and Przedborski, S. 2003. Nitric oxide and reactive oxygen species in parkinson's disease. *IUBMB Life* **55**, 329-335.
38. Van Eldik, L. J., Thompson, W. L., Ralay Ranaivo, H., Behanna, H. A. and Martin Watterson, D. 2007. Glia proin-

flammatory cytokine upregulation as a therapeutic target for neurodegenerative diseases: Function-based and target-based discovery approaches. *Int. Rev. Neurobiol.* **82**, 277-296.
39. Wakabayashi, C., Murakami, K., Hasegawa, H., Murata, J. and Saiki, I. 1998. An intestinal bacterial metabolite of ginseng protopanaxadiol saponins has the ability to induce apoptosis in tumor cells. *Biochem. Biophys. Res. Commun.* **246**, 725-730.
40. Wei, T., Chen, C., Hou, J., Xin, W. and Mori, A. 2000. Nitric oxide induces oxidative stress and apoptosis in neuronal cells. *Biochim. Biophys. Acta* **1498**, 72-79.
41. Yang, Y., Yang, W. S., Yu, T., Sung, G. H., Park, K. W., Yoon, K., Son, Y. J., Hwang, H., Kwak, Y. S., Lee, C. M., Rhee, M. H., Kim, J. H. and Cho, J. Y. 2014. ATF-2/CREB/IRF-3-targeted anti-inflammatory activity of Korean red ginseng water extract. *J. Ethnopharmacol.* **154**, 218-228.

초록 : 홍삼추출액은 lipoteichoic acid로 자극된 소교세포에서 Akt 및 MAPK 의존적으로 heme oxygenase-1 발현을 유도함으로써 NO 생성을 억제함

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생삼을 쪄서 건조시킨 홍삼은 전통적으로 사용되고 있는 약재로서 면역, 내분비 및 중추신경계 작용을 증진시키며 염증을 억제하는 효과가 있는 것으로 알려져 있다. 본 연구에서는 그람 양성균의 세포벽 성분인 lipoteichoic acid (LTA)에 의한 염증반응에 홍삼추출액(RGE)이 항염증 효과를 가지는지 관찰하고 그 작용 기전을 연구하였다. BV-2 소교세포에서 RGE는 세포에 독성을 유도하지 않으면서 LTA로 인한 nitric oxide (NO)의 생성과 inducible nitric oxide synthase (iNOS) 발현을 억제하였으며, NF-kB p65의 핵으로의 이동과 IkB-a의 분해 또한 억제하였다. 한편, RGE는 농도의존적으로 heme oxygenase-1 (HO-1)의 발현을 유도하였으며, HO-1 siRNA를 처리했을 때는 RGE가 iNOS의 발현을 억제하지 못하였다. RGE는 HO-1의 발현에 관여하는 전사인자인 nuclear factor E2-related factor 2 (Nrf2)를 핵으로 이동을 촉진시켰다. 또한 RGE에 의한 HO-1의 발현은 phosphatidylinositol-3-kinase (PI-3K) 및 MAPK 억제제에 의해 감소되었으며, RGE가 Akt와 ERK, p38, JNK의 인산화를 유도하였다. 이상의 결과를 종합해보면, RGE는 PI-3K/Akt 및 ERK, p38, JNK 신호전달과정을 통해 HO-1의 발현을 유도함으로써 NO와 같은 염증매개물질의 생성을 억제한다는 것을 알 수 있다. 그러므로 홍삼추출액은 그람 양성균에 의한 신경염증과 염증관련 신경계 질환의 치료제로서 사용될 수 있을 것이라 사료된다.