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American ginseng preferentially suppresses STAT/iNOS signaling in activated macrophages

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Abstract

Aim of the study—Ginseng has been used as general tonic for thousands of years in Asia and becomes a popular herbal medicine all over the world. However, the cellular and molecular mechanisms underlying its benefit effects are less explored. Thus, we investigated the effect of a crude extract from *Panax quinquefolius* (American ginseng) on suppression of pro-inflammatory responses in macrophages with a focus on signal transducer and activator of transcription (STAT) signaling.

Materials and methods—The crude extract of American ginseng that was supplied by the National Research Council of Canada, Institute for National Measurement Standards (NRCC-INMS) was freshly solvated in Dulbecco's Modified Eagle Medium (DMEM) prior to each experiment. RAW264.7 cells, a murine macrophage cell line, were exposed to lipopolysaccharide (LPS) to induce inflammatory responses such as expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2). Proteins were measured by Western blot and mRNA expression was determined by quantitative real time PCR (Q-PCR). Activator protein 1 (AP-1)-, nuclear factor-κB (NF-κB)- and STAT-mediated transcriptional activities were investigated using luciferase reporter constructs.

Results—American ginseng inhibited LPS-induced iNOS expression; however, it did not affect LPS-induced COX2 expression. While American ginseng had no impact on LPS-induced activation of AP-1 or NF-κB pathways, it dramatically inhibited LPS-induced activation of STAT signaling. Moreover, American ginseng and AG490, an inhibitor of STAT cascade, synergistically suppressed the LPS-induced iNOS expression.

Conclusion—American ginseng selectively inhibits the expression of iNOS via suppression of STAT cascade but not NF-κB and AP1 pathways in inflamed macrophages. Such a preferential suppression of STAT/iNOS cascade by American ginseng might have therapeutic potential for inflammatory diseases with over-activation of iNOS.

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Keywords

Panax. quinquefolius; American ginseng; STAT; Macrophages; Inflammation; iNOS; COX2

1. Introduction

Ginseng is the root of *genus Panax;* including *Panax ginseng* (Chinese and Korean ginseng), *P. notoginseng* (Chinese Sanqi ginseng), and *P. quinquefolius* (American ginseng). The name ginseng comes from the Chinese words “Ren Sheng,” meaning “man-herb,” because of the humanoid shape of the root or rhizome of the plant. Carl Anton Meyer, a Russian botanist, named it Panax ginseng C. A. Meyer, in 1843 (Gillis, 1997). The genus name Panax means “All-Healing” or “Cure All” in Greek, which describes the traditional belief that ginseng has properties to heal all aspects of the body. Ginseng has been used as a general tonic for thousands of years in Asian countries, and has become a popular herbal medicine all over the world. Recent research shows that regular use of ginseng is helpful in the treatment of Alzheimer’s disease, diabetes, aging, immune disorders, cancer and cardiovascular disease (Attele *et al.*, 1999; Hofseth *et al.*, 2007; Radad *et al.*, 2006; Wang *et al.*, 2007; Xie *et al.*, 2005). However, the therapeutic efficacy of ginseng has not been established. The cellular and molecular mechanisms by which ginsengs induce pleiotropic biological actions remain largely unknown.

It is well known that chronic inflammation contributes to the pathogenesis of many human diseases such as cancer, diabetes, hypertension and atherosclerosis. Of interest, several lines of evidence have revealed that ginseng regulates inflammatory responses (Hofseth *et al.*, 2007; Spelman *et al.*, 2006). Although a few studies show that ginseng may trigger inflammatory responses, most of the reports demonstrate that ginseng exerts anti-inflammatory effects. The reasons for these discrepancies are unclear, perhaps because the content of ginseng root or root extracts can differ, depending on the method of extraction and subsequent treatment. Nevertheless, the underlying molecular mechanisms are less explored.

Herein, we report that *P. quinquefolius* (American ginseng) suppresses lipopolysaccharide (LPS)-induced activation of signal transducer and activator of transcription (STAT) pathway, leading to a preferential inhibition of inducible nitric oxide synthase (iNOS) expression in macrophages.

2. Materials and methods

2.1. Materials

American ginseng extract was supplied by the National Research Council of Canada, Institute for National Measurement Standards (NRCC-INMS). The ginseng roots were processed by Canadian Phytopharmaceutical Corporation (Richmond, British Columbia, Canada). Following grinding to pass 80 mesh, 35 kg of the root material was extracted with aqueous ethanol (75% ethanol/25% H_2O) in a re-circulating filter extraction system for 4 hours at a temperature of 60°C under vacuum. The ratio of solvent to root was 8:1 (v/w). After extraction, the filtrate was partially dried in-vacuo to yield a concentrated extract, then 2.8 kg of maltodextrin (40% of final weight) was blended as a support and the resultant slurry spray dried to yield 7 kg of free flowing powder. The total ginsenoside content as the sum of Rg1, Re, Rb1, Rc, Rb2 and Rd of the finished materials was determined by Canadian Phytopharmaceutical Corporation with HPLC-UV against pure standards, and confirmed by HPLC-MS at the National Research Council of Canada. The final, powder form of American ginseng extract supplied by Canadian Phytopharmaceutical Corp. contains (w/w) 10% ginsenosides (Rg1, Re, Rb1, Rc, Rb2 and Rd), 2% (additional ginsenosides made up of (F11, Ro, isomers of Rd, and traces of malonyl ginsenosides) 40% of maltodextrin derived from

hydrolysed corn starch. The remaining 48% of the powder is made up of ginseng root derived polysaccharides/ligosaccharides and proteins and up to 5% of moisture). The lot utilized in this study was screened and found to comply with standards set (e.g., NSF) for heavy metals and contaminants in dietary supplements and is periodically tested by NRCC-INMS to confirm stability of the ginsenoside content. Solutions of American ginseng, at different concentrations, were freshly prepared before each experiment. Reporter plasmids of pISRE-TA-Luc and pNFκB-TA-Luc for monitoring STAT and nuclear factor k (NF-κB) transcriptional activity respectively were purchased from Clontech (Palo Alto, CA, USA.) Reporter plasmid of pAP-1-TA-Luc for activator protein(AP)-1 transcriptional activity was purchased from Stratagene (Cedar Creek, Texas, USA). pISRE-TA-Luc contains five copies of interferon-γ stimulated response element (ISRE) enhancer elements, located upstream of minimal TA promoter-driven firefly luciferase (Luc) reporter gene. Similarly, pNFκB-TA-Luc or pAP-1-TA-Luc contains multiple copies of the NFκB consensus sequence or AP-1 binding sites, respectively. Upon binding of the STAT1 and STAT2 heterodimer to the *cis*-acting ISRE enhancer element, the NF-κB or AP-1 to their binding sites, the transcription is induced and the reporter gene is activated. LPS and AG490 were purchased from Sigma-Aldrich (St. Luis, MO, USA.

2.2. Cell culture, transfection and reporter gene luciferase assay

RAW264.7 (American Type Culture Collection) cells were cultured in DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum. Cell viability was determined by the monitoring of release of the cytoplasmic enzyme lactate dehydrogenase (LDH) utilizing a cytotoxicity detection kit (Roche Applied Science). Before the start of each experiment, cells were cultured with serum-free DMEM for 24 h to induce a quiescent status and followed various treatments as previously reported (Cieslik *et al.*, 2002; Ichikawa *et al.*, 2008; Kim *et al.*, 2006b). Serum starvation for 48 h did not exert apparent cytotoxic effect in RAW264.7 cells. Transcriptional activity of NF-κB, AP-1 and STAT was determined by measurement of luciferase activities in RAW264.7 cells that were transfected with their reporter plasmids as previous described (Ichikawa *et al.*, 2008). Briefly, RAW264.7 cells were transfected with pNFκB-TA-Luc or pAP-1-TA-Luc or pISRE-TA-Luc together with pRL-TK (Promega) for normalization of transfection efficiency using FuGENE 6 transfection reagent (Roche Diagnostics, Indianapolis, IN). The NF-κB, AP-1 or STAT transcriptional activity was determined using a dual luciferase assay kit (Promega, Madison, WI) according to the manufacturer's instructions with a luminometer (Victor II; Perkin-Elmer, Wellesley, MA).

2.3. Quantitative real-time PCR (Q-PCR)

Expression of iNOS and cyclooxygenase 2 (COX2) mRNAs was assessed by Q-PCR as previously described (Ichikawa *et al.*, 2008). Briefly, total RNA from cell pellets was extracted using RNeasy kits (Qiagen Inc., Valencia, CA), and reverse transcription reactions (Advantage RT for PCR kit; Clontech) were performed with 0.5–1 μg of DNase I (Qiagen)-treated RNA. Q-PCRs were carried out using the Mastercycler EP Realplex (Eppendorf, Westbury, NY) and the SYBR green I kits (Roche Applied Science) according to the manufacturer's recommendations. Forward primer (5′-GAGGCCGCATGAGCTTGGTGTTT-3′) and reverse primer (5′-GGGGGTTGCATTTCGCTGTCTCC-3′) were used for PCR amplification of iNOS to yield a 511-bp product. Forward primer (5′-CCTGCTGCCCGACACCTTCAACAT-3′) and reverse primer (5′-CAGCAACCCGGCCAGCAATCT-3′) were used for PCR amplification of COX2 to yield a 140-bp product. Cycle numbers obtained at the log-linear phase of the reaction were plotted against a standard curve prepared with serially diluted control samples. Expression levels of iNOS or COX2 were normalized by concurrent measurement of GAPDH mRNA levels.

2.4. Western blot analysis

Western blot analysis was performed as previously described (Villacorta *et al.*, 2007). Briefly, cells treated under different experimental conditions were washed quickly with ice-cold PBS containing 1 mM Na_3VO_4, frozen in liquid nitrogen, scraped off, and lysed in Nonidet P-40 lysis buffer (1% Nonidet P-40, 25 mM HEPES (pH 7.5), 50 mM NaCl, 50 mM NaF, 5 mM EDTA, 10 nM okadaic acid, 1 mM sodium orthoavanadate, 1 mM phenylmethylsulfonyl fluoride, and 10 μM aprotinin) for 10–15 min on ice. Insoluble material was removed by centrifugation at 14,000 × *g* for 20 min at 4 °C. Protein concentration was measured in the cleared supernatant via a protein assay kit (Bio-Rad). An equal amount of cytosolic fractions was subjected to Western blot analysis using antibodies of phospho-STAT1 (pSTAT1) (Tyr-701), STAT1, p-ERK, p-p38, p-IκBα (Ser32/36) and p-JNK (Cell Signaling Technology, Inc.); pSTAT2 (Tyr-689)(Millipore, Temecula, CA); c-Jun, p-c-Jin, ERK, p38, iκB, STAT2 and GAPDH (Santa Cruz Biotechnology, Inc., Santa Cruz, CA); COX2 and iNOS (BD Biosciences, San Jose, CA). Densitometric analysis was performed by using an image scanner (EPSON GT-8000) and NIH image software.

Statistics

2.5. Values are expressed as mean ± SD in the text and figures. The data were analyzed using ANOVA with the Newman-Keuls' test unless specified. Values of $P < 0.05$ were considered to be statistically significant.

3. Results

American ginseng selectively inhibits LPS-induced iNOS

As the evidential discrepancies on the role of ginseng in regulating inflammatory responses (Hofseth *et al.*, 2007), we first examined effect of the American ginseng extract supplied by NRCC-INMS on LPS-induced inflammatory responses with a focus on the expression of iNOS and COX2 in RAW264.7 macrophages. To optimize our experimental system, we examined impact of the American ginseng extract on cell viability in RAW264 macrophages. We observed that 95% of RAW264.7 cells survived the serum starvation and American ginseng did not affect the viability prior to a dose of 100 μg/ml (Fig. 1A) Thus the dose of American ginseng that we used thereafter was up to 50 μg/ml. While the LPS-induced iNOS protein expression was dramatically inhibited by American ginseng, the LPS-induced COX2 protein was hardly affected (Fig. 1B). Of interest, American ginseng alone slightly but significantly induced the expression of iNOS and COX2 proteins in a dose-dependent manner (Fig. 1C). These observations were further confirmed by the results that American ginseng preferentially inhibited iNOS expression at mRNA level in a dose-dependent manner while it alone induced slight increases in the expression of iNOS and COX2 mRNAs (Fig. 1D).

Molecular mechanism by which American ginseng suppresses LPS-induced iNOS expression

Next, we mechanistically analyzed the American ginseng-mediated regulation of iNOS and COX2 expression in LPS-inflamed RAW264.7 cells. Because iNOS and COX2 gene expressions are regulated mainly at the transcriptional level through the activation of mitogen-activated protein kinases (MAPKs), AP-1, NF-κB, and STATs (Fujihara *et al.*, 2003; Ichikawa *et al.*, 2008; Janabi, 2002; Kim *et al.*, 2006a; Kristof *et al.*, 2001; Xie *et al.*, 1993), we examined the effect of American ginseng on LPS-induced activation of these signaling pathways in RAW264.7 macrophages.

Effect of American ginseng on MAKP pathway—LPS activated MAPKs including ERK, p38 and JNK as well as c-Jun phosphorylation and AP-1-operated transcription in

macrophages as described elsewhere (Fujihara *et al.*, 2003); however, American ginseng had no impact on the LPS-induced action of MAKP and AP-1 transcriptional activity in RAW264.7 cells (Fig. 2 and 3A).

Effect of American ginseng on NF-κB pathway—In resting cells, NF-κB is typically sequestered in the cytoplasm by association with an inhibitory protein, IκB. In response to various stimuli, IκB kinase is activated and phosphorylates IκB on two serine residues, resulting in IκB ubiquitination, degradation by the proteasome, and facilitation of NF-κB migration into the nucleus to mediate the expression of inflammatory response genes such as iNOS and COX2 (Ghosh *et al.*, 1998). As shown in Fig. 3B, LPS robustly activated NF-κB pathway that is evidenced by the enhancement of IκB phosphorylation, degradation and the subsequent activation of NF-κB transcriptional activity in RAW264.7 cells; however, American ginseng did not affect the LPS-induced activation of NF-κB pathway. Of note, American ginseng alone slightly activated NF-κB pathway in RAW264.7 cells (Fig. 3B).

Effect of American ginseng on STAT signaling—To determine whether American ginseng interrupts LPS-driven STAT pathway that is critical for iNOS expression in macrophages (Ichikawa *et al.*, 2008), we examined the effect of American ginseng on LPS-induced STAT1 and STAT2 signaling in RAW264.7 cells. American ginseng completely blocked the phosphorylation of STAT1 and STAT2 and the subsequent STAT1- and STAT2-operated transcriptional activity by LPS (Fig. 4A and B). In addition, AG490, a Jak2 kinase inhibitor, dose-dependently inhibited LPS-induced iNOS expression; however, it did not affect LPS-induced COX expression (Fig. 4C). Importantly, AG490 and American ginseng synergistically inhibited iNOS expression induced by LPS (Fig. 4D).

4. Discussion

Ginseng has been estimated to be the second top-selling herbal supplement. In the US, annual sales of ginseng were $62 million in 2006. The public enthusiasm for ginseng has been at least partly inspired by the growing evidence of ginseng-mediated anti-inflammatory and/or immunomodulatory actions (Hofseth *et al.*, 2007; Spelman *et al.*, 2006). In the present study, we have demonstrated that American ginseng extract supplied by the National Research Council of Canada, Institute for National Measurement Standards (NRCC-INMS) induces relative low levels of iNOS and COX2 expressions via the activating of IκB/NF-κB pathway in quiescent macrophages; however, it could also preferentially inhibit iNOS expression by the suppressing of STAT signaling in inflamed macrophages. Thus, a nature of the American ginseng-mediated regulation of iNOS and COX2 expression is not a simply stimulatory or inhibitory action, but a unique modulatory process, leading to the facilitating of macrophage function.

It has been reported that crude extract of ginseng inhibits LPS-induced iNOS expression (Seo *et al.*, 2005) whereas it alone induces iNOS expression in RAW264.7 macrophages (Friedl *et al.*, 2001). In addition, extract of ginsenosides from heat-processed ginseng inhibits 12-O-tetradecanoylphotbol-13-acetate (TPA)-induced COX2 expression in human breast epithelial cells as well as TPA-induced edema in mouse skin associated with down-regulation of COX expression (Park *et al.*, 2007; Surh *et al.*, 2002). However, it has also been documented that ginsenoside Rd *per se* induces COX2 expression in RAW264.7 macrophages (Jeong *et al.*, 2007). Accordingly, our results demonstrate that American ginseng from NRCC-INMS preferentially suppresses LPS-induced iNOS expression in activated macrophages while it induces relative low levels of iNOS and COX2 expressions in quiescent macrophages (Fig. 1). These results reveal that the content in crude extracts of ginseng is linked with their biological actions. Because of the unique feature of American ginseng-mediated regulation of iNOS and COX2 expression, to further elucidate its underlying molecular mechanism will provide a novel

insight into the understanding of ginseng-mediated anti-inflammatory and/or immunomodulatory actions.

Although several signaling pathways contribute to LPS-induced iNOS and COX2 expression as aforementioned, American ginseng might interfere with specific the signaling cascades in macrophages, thereby regulating iNOS and COX expression. In RAW264.7 macrophages, a recent study has shown that not ERK and p38 kinase but JNK and NF-κB cascases mediate LPS-induced iNOS and COX2 expression (Tsoyi *et al.*, 2008). In the present study, we demonstrated that LPS activated MAPK cascades including ERK, JNK and p38; however, American ginseng affected none of these cascades in LPS-inflamed RAW264.7 cells (Fig. 2). Moreover, American ginseng did not alter either basal or LPS-induced phosphorylation of c-Jun, a downstream molecule of JNK, as well as AP-1 transcriptional activity, a subsequent event of c-Jun activation/phosphorylation (Shaulian *et al.*, 2002)(Fig. 3A). These results clearly wipe out the possibility that American ginseng regulates iNOS or COX2 expression via MAPK EEK, p38 and JNK cascades in RAW264.7 macrophages. On the other hand, while American ginseng had no impact on LPS-induced IκBα phosphorylation, degradation, and subsequent NF-κB transcriptional activation in RAW264.7 cells, it alone did induce slight activation of the IκBα/NF-κB pathway (Fig. 3B). These results suggest that American ginseng alone induces iNOS and COX2 expression via activation of IκB/NF-κB pathway in macrophages as previously described (Friedl *et al.*, 2001); however, it could inhibits iNOS expression via a mechanism that dose not involve NF-kB pathway. Taken together, we demonstrate that American ginseng suppresses iNOS expression via a mechanism independent of MAPK/AP-1 and IκB/NF-κB pathways in macrophages.

Since we and others have demonstrated that LPS activates STAT/iNOS signaling and STAT signaling is essential for LPS-induced iNOS expression rather than COX2 expression in RAW264.7 macrophages (Ichikawa *et al.*, 2008; Tsoyi *et al.*, 2008), we explored whether or not American ginseng suppresses STAT/iNOS in RAW264.7 cells. As shown in Figs. 4A and B, American ginseng dramatically suppressed LPS-induced phosphorylation of STAT1 and STAT2 as well as heterodimer of STAT1 and STAT2-driven transcriptional activity. In addition, we confirmed that inhibition of STAT activation by Jak2 inhibitor AG490 selectively attenuates iNOS expression in LPS-inflamed RAW264.7 cells (Tsoyi *et al.*, 2008)(Fig. 4C). Importantly, American ginseng and AG490 exerted a synergistic inhibition of the LPS-induced both STAT1 phosphorylation and iNOS expression (Fig. 4D), suggesting that American ginseng preferential inhibits STAT signaling, leading to down-regulation of iNOS expression in the LPS-inflamed macrophages.

Our finding that American ginseng modulates a proper expression of iNOS and COX2 is especially of clinical interest. In fact, iNOS and COX2 are important regulators of inflammatory responses in various tissues and organs, and play important roles in the pathogenesis of human disease (Hui, 2008; Mathrani *et al.*, 2007; Salinas *et al.*, 2007). Emerging evidence has also revealed that a delicate cross-talk between COXs and NOSs plays a critical role in the control of inflammation and other pathophysiological events (Cuzzocrea *et al.*, 2007; Mariotto *et al.*, 2007). A temporal dynamic and quantitative expression of iNOS and COX2 is essential for the control of immune balance and proper inflammatory responses, thereby contributing to the maintenance of functional integrity of tissues and organs. In this context, the unique modulatory effect of American ginseng on the control of proper iNOS and COX2 expression might represent a novel mechanism to strengthen adaptive immunity and inflammatory resolution.

It has been established that COX2 is distinctively coupled with the terminal prostaglandin (PG) synthases including PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2, and thromboxane synthases to generate different PGs in cell- and tissue-dependent manner (Ueno *et al.*, 2005). Induction of membrane-

bound PGES (mPGES)-1 is induced by pro-inflammatory stimuli including LPS and is down-regulated by anti-inflammatory glucocorticoid. COX2/mPGES-1/PGE_2 coupling leads to development of inflammatory disease such as arthritis. In contrast, COX2/PGIS/PGI_2 coupling exerts beneficial effects in cardiovascular system. Interestingly, it has been demonstrated that ginseng extract activates glucocorticoid receptor (Chung *et al.*, 1998; Leung *et al.*, 2006a; Leung *et al.*, 2007; Leung *et al.*, 2006b; Ling *et al.*, 2005). Therefore, it is possible that American ginseng affects COX2/mPGES-1/PGE_2 coupling via activation of glucocorticoid receptor, resulting inflammatory resolution rather than inflammation in macrophages. Accordingly, whether American ginseng could facilitate special coupling between COX2 and anti-inflammatory terminal prostanoid synthases in macrophages deserves further investigation.

In summary, we have demonstrated that preferential suppression of STAT cascade as a novel mechanism contributing to the anti-inflammatory activity of American ginseng in macrophages. Further characterization of American ginseng-mediated signal transduction pathways will untwist the molecular bases by which ginseng acts as a general tonic or perhaps as a medicine for the treatment of inflammatory disease.

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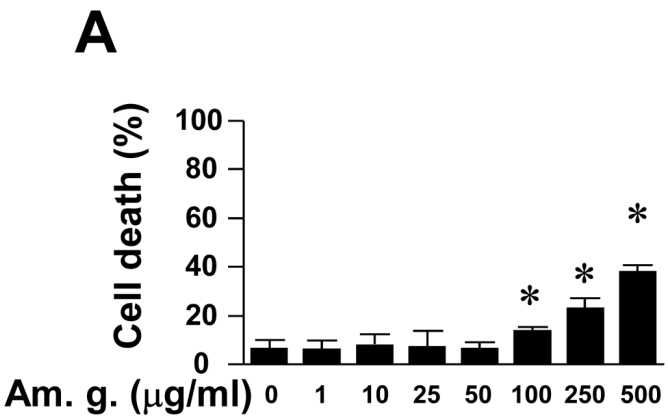
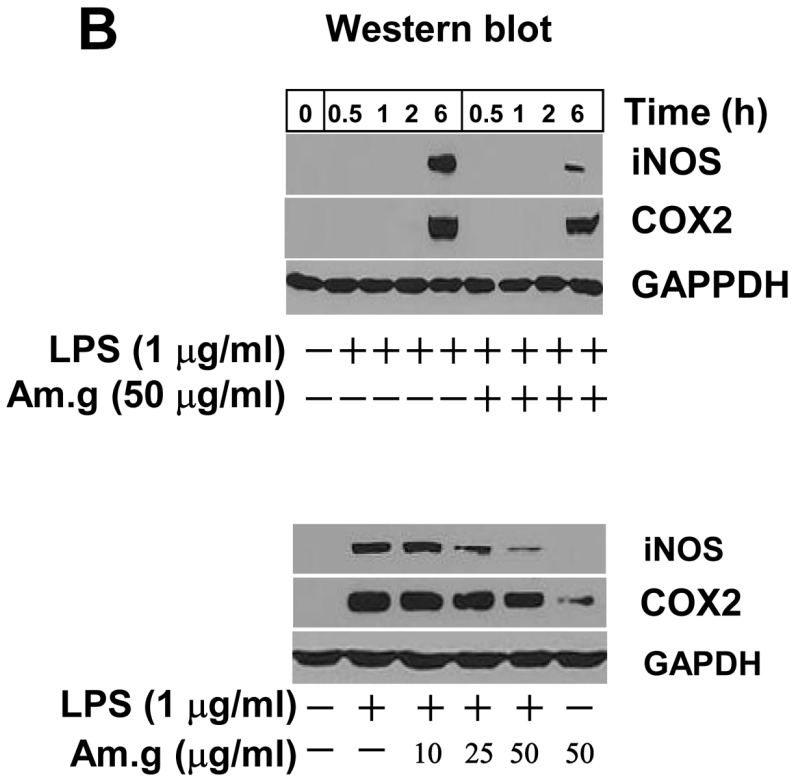




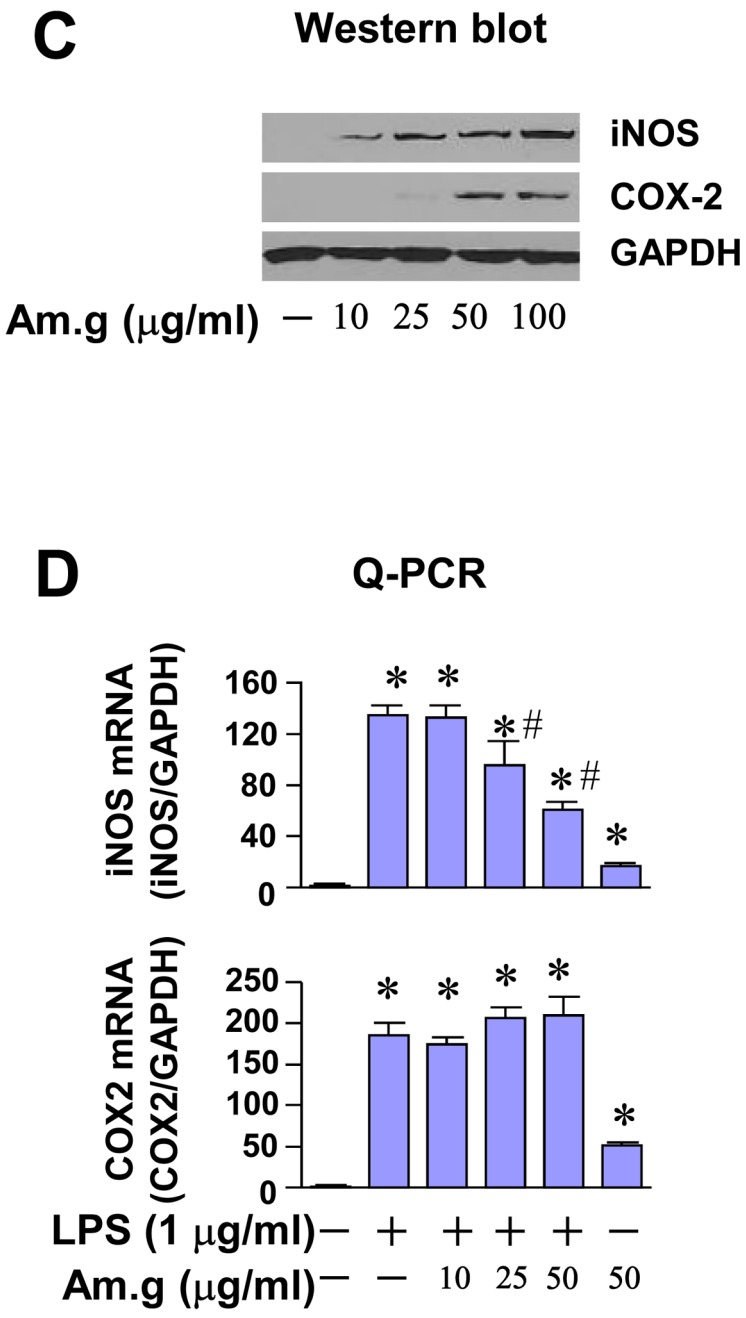


Fig. 1.

Effect of American ginseng on LPS-induced expression of iNOS and COX2 in RAW264.7 cells. (**A**) RAW264.7 cells were cultured in serum-free DMEM with or without crude extract from root of American ginseng (Am.g) for 48 h. Cell viability was assessed by a cell death kit as described in “Method”. (p*<0.05 vs control). (**B**) RAW264.7 cells were cultured in serum-free DMEM for 24 h to induce a quiescent status, and then treated with LPS and Am.g as indicated. Protein levels of iNOS and COX2 were determined by Western blot. Results are representatives of three separate experiments. (**C**) The quiescent RAW264.7 cells were stimulated with Am.g alone as indicated for 6 h and then protein levels of iNOS and COX2 were determined by Western blot. Results are representatives of three separate experiments.

(**D**) The quiescent RAW264.7 cells were treated with LPS and Ag for 6 h as indicated. Upper panel was representatives of Western blot analysis of iNOS and COX2 from three separate experiments. Lower panel was quantitative analysis of mRNA levels of iNOS and COX2 by Q-PCR (n=6, *$p<0.05$ vs control (−); #$p<0.05$ vs LPS (+))

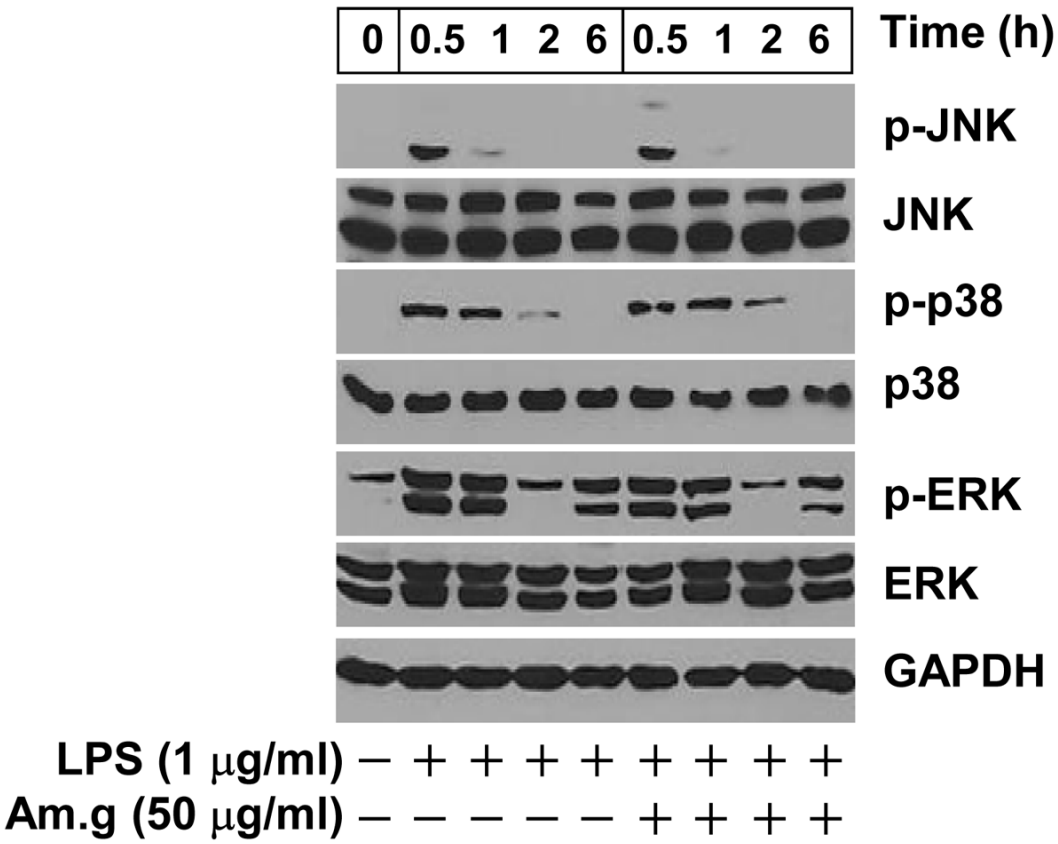
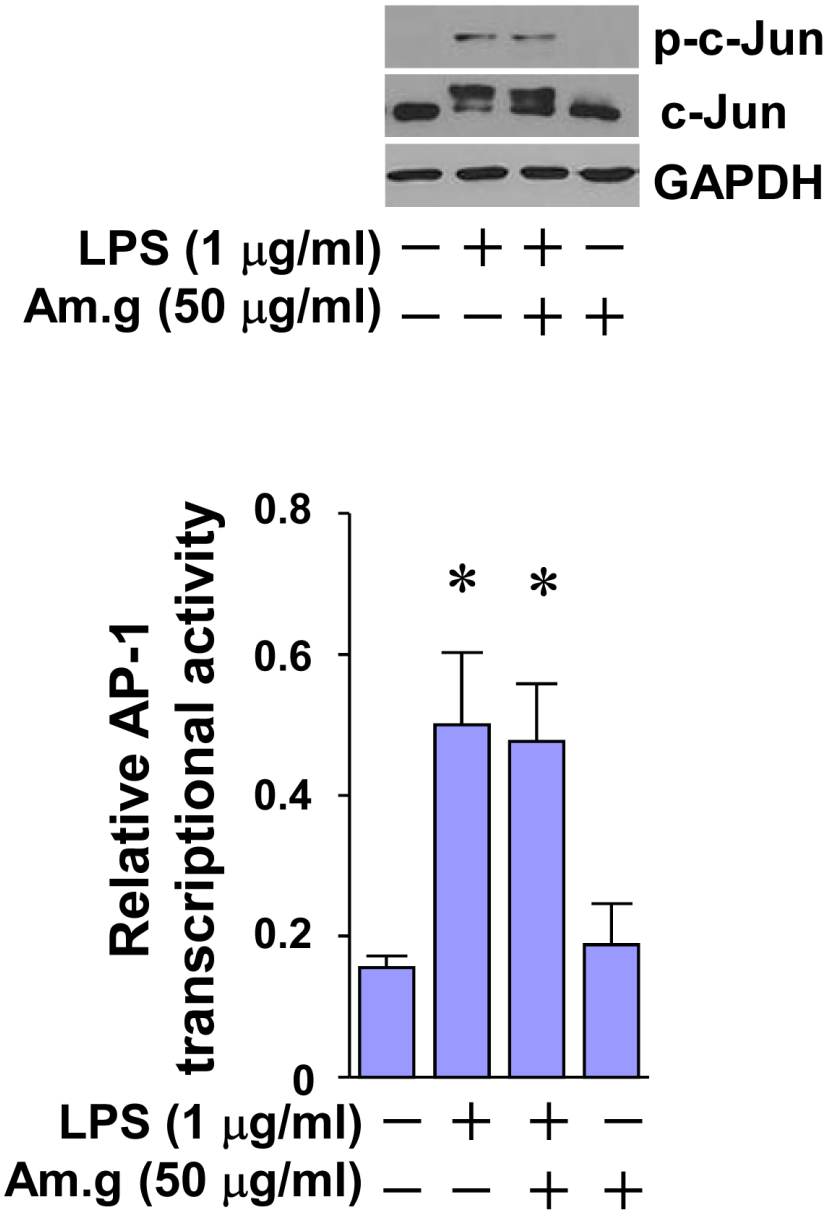


Fig. 2.

Effect of American ginseng on LPS-induced activation of MAPKs in RAW264.7 cells. Quiescent cells were treated with LPS and a crude extract from root of American ginseng (Am.g) as indicated. Cells were lysed and cytosolic fractions were subjected to Western blot analysis. Results are representatives from three separate experiments.



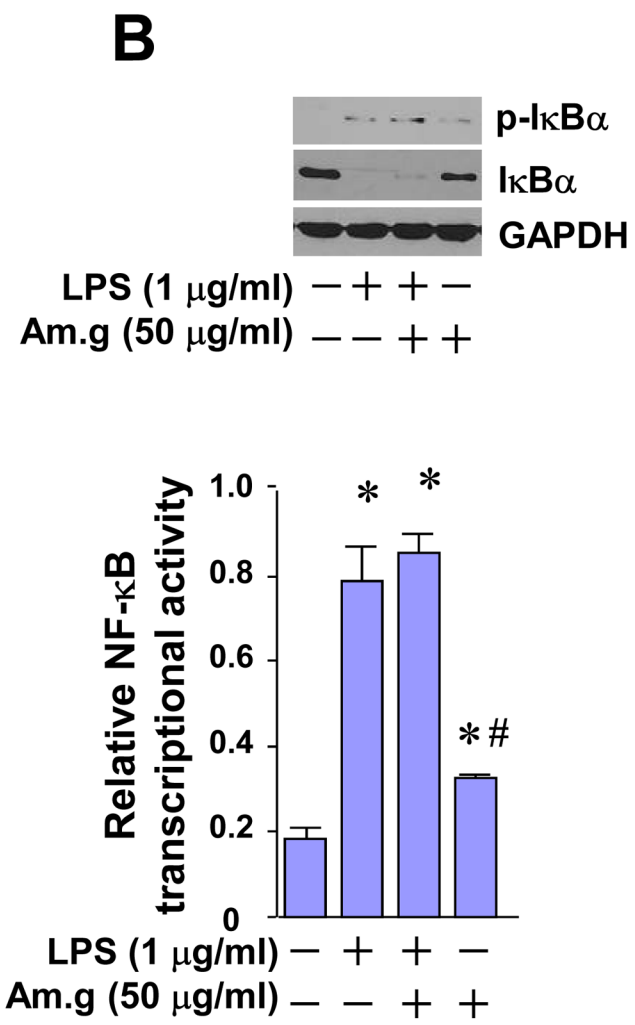
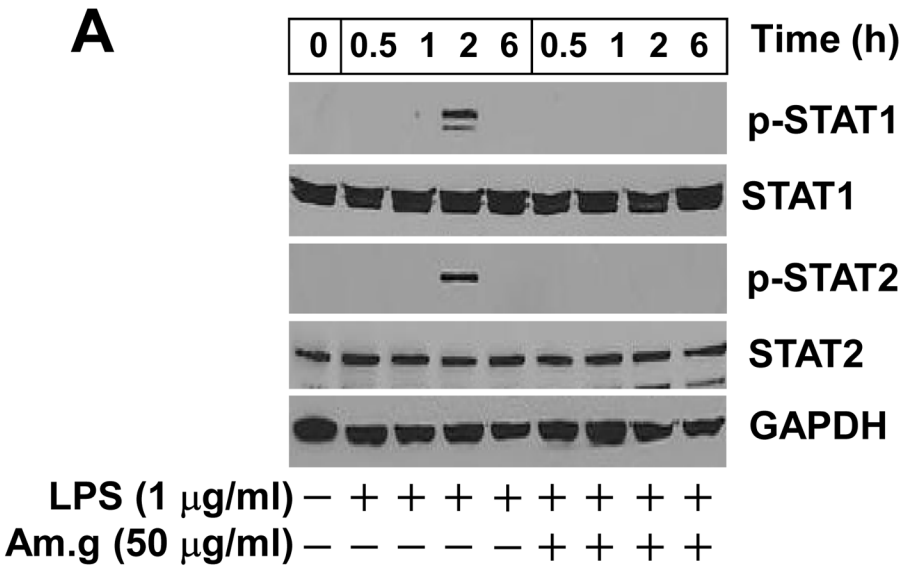
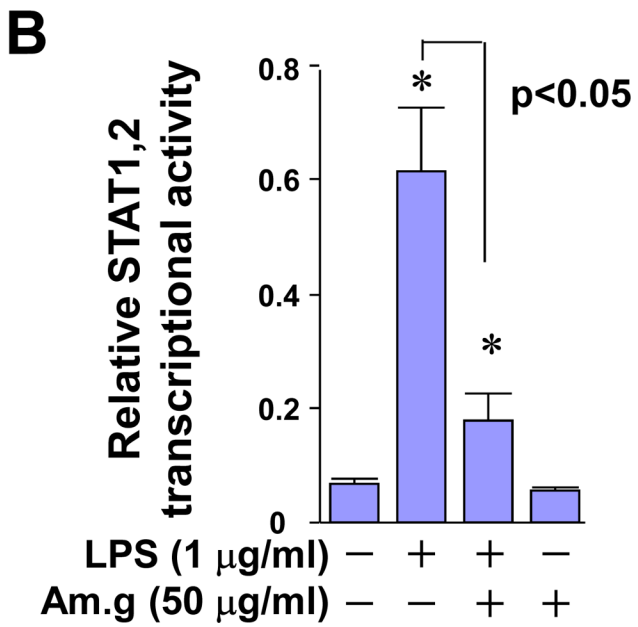


Fig. 3.

Effect of American ginseng on LPS-induced activation of AP-1 and NF-κB cascades in RAW264.7 cells. (**A**) *Upper panel:* Quiescent cells were treated with LPS and a crude extract from root of American ginseng (Am.g) for 3 min as indicated. Phosphorylation of c-Jun and total c-Jun were assessed by Western blot analysis. Results are representatives from three separate experiments. *Lower panel:* Cells were transfected with AP-1 reporter constructs as well as internal control plasmids of pRL-TK, and then cultured in serum-free DMEM for 24 h before stimulation with LPS and Am.g for 4 h as indicated. AP-1 transcriptional activity was assessed by a duel luciferase assay as described in "Methods" (n=6, *p<0.05 vs control (−)). (**B**) *Upper panel:* Quiescent cells were treated with LPS and a crude extract from root of American ginseng (Am.g) for 15 min as indicated. Phosphorylation and protein levels of IκB were determined by Western blot. *Lower panel:* Cells transfected with NF-κB reporter constructs and as well as internal control plasmids of pRL-TK, stimulated with LPS and Am.g for 6 h, and then NF-κB transcriptional activity was assessed as a duel luciferase assay as described in "Methods" (n=6, *p<0.05 vs control (−), #p<0.05 vs LPS(+)).





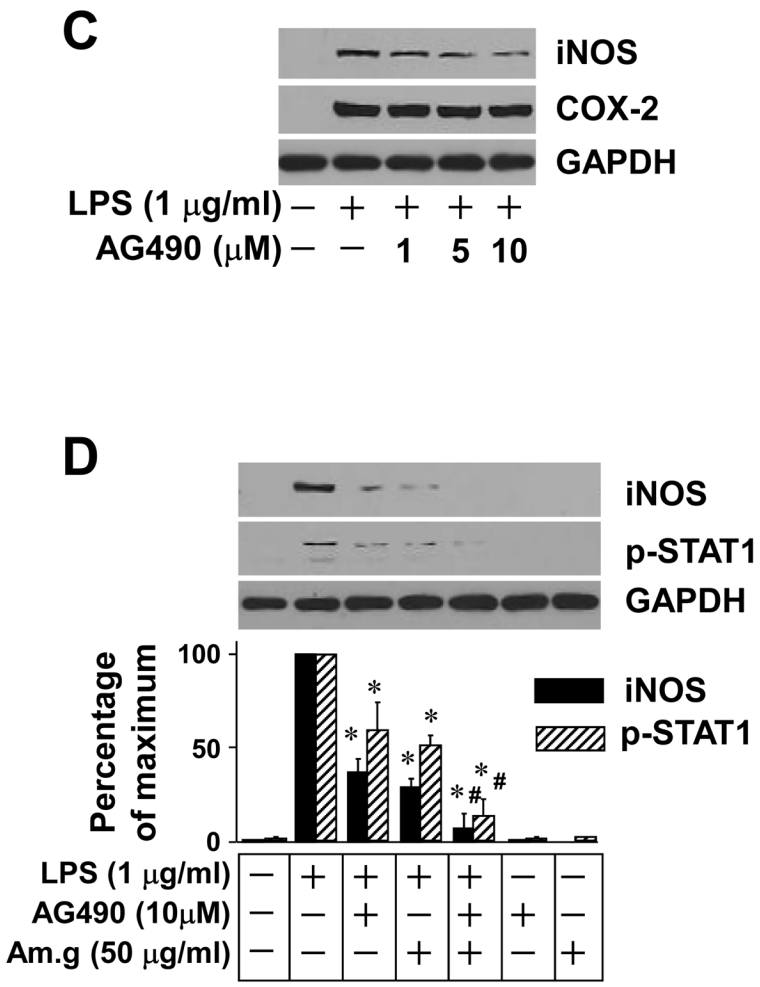


Fig. 4.

Effect of American ginseng on STAT signaling in RAW264.7 cells. (**A**) Quiescent cells were stimulated as indicated. Phosphorylation of STAT1 and 2 as well as total STAT1 and 2 were assessed by Western blot analysis. (**B**) Cells were transfected with STAT reporter constructs as well as internal control plasmids of pRL-TK, stimulated for 6 h, and then followed with the assay of STAT1and 2 transcriptional activity as described in "Method" (n=6, *p<0.05 vs control (−)). (**C**) Quiescent cells were stimulated for 6 h. Protein levels of iNOS and COX2 were determined by Western blot analysis. (**D**) Cells were treated with different stimuli as indicated. Phosphorylation of STAT1 was assessed 2 h after the stimulation. Protein levels of iNOS were determined 6 h after the stimulation. Lower panel is densitometric analysis of iNOS and p-STAT1 immunoblots (n=4, *p<0.05 vs LPS (−); #p<0.05 vs LPS+Am.g. or LPS +AG490). The density of iNOS expression or STAT1 phosphorylation induced by LPS alone was set as the maximal increase (100%).