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Ginsenosides compound K and Rh_2 inhibit tumor necrosis factor-α-induced activation of the NF-κB and JNK pathways in human astroglial cells

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Abstract

Ginsenosides, the main component of Panax ginseng, have been known for the anti-inflammatory and anti-proliferative activities. In this study, we investigated the molecular mechanisms responsible for the anti-inflammatory effects of ginsenosides on activated astroglial cells. Among 13 different ginsenosides, intestinal bacterial metabolites Rh_2 and compound K (C-K) showed a significant inhibitory effect on tumor necrosis factor-α (TNF-α)-induced expression of intercellular adhesion molecule-1 in human astroglial cells. Pretreatment with C-K or Rh_2 suppressed TNF-α-induced phosphorylation of IκBα kinase and the subsequent phosphorylation and degradation of IκBα. Additionally, the same treatment inhibited TNF-α-induced phosphorylation of MKK4 and the subsequent activation of the JNK-AP-1 pathway. The inhibitory effect of ginsenosides on TNF-α-induced activation of the NF-κB and JNK pathways was not observed in human monocytic U937 cells. These results collectively indicate that ginsenoside metabolites C-K and Rh_2 exert anti-inflammatory effects by the inhibition of both NF-κB and JNK pathways in a cell-specific manner.

Keywords: Glia; Inflammation; Ginsenosides; NF-κB; JNK; Tumor necrosis factor

Ginseng has long been used as an herbal drug in traditional oriental medicine and is now used extensively as a general health supplement. Ginsenosides, major pharmaceutical active ingredients of ginseng, are shown to have a broad range of biological activities such as anti-oxidation, anti-inflammation, and anti-proliferation [16]. Several ginsenosides are also reported to have a neuroprotective effect, even though the exact molecular mechanisms have not been determined [11,12].

Astrocytes, major glial cells in the central nervous system, play neuroprotective roles by maintaining mechanical and chemical homeostasis, blood–brain barriers, and immune suppression under physiologic conditions. Excessive activation of astroglial cells, however, is regarded as a key pathogenic event leading to uncontrolled neuroinflammation and subsequent neuronal loss in various clinical settings, including neurodegeneration. Therefore, attenuation of astroglial hyperactivation is thought to be a therapeutic target for treatment of such neurodegenerative disorders as Alzheimer's disease [24]. Several ginsenosides (including Rg_1, Rg_3, and Rh_2) are reported to be effective in acute ischemic brain injury and chronic neuroinflammation through the suppression of the excessive activation of microglial cells [2,8]. Even though the neuroprotective and anti-inflammatory effect of ginsenosides are well documented, the molecular mechanisms responsible for these biological activities are yet to be determined. In this study, we investigated the anti-inflammatory effect of ginsenosides on reactive astroglial cells and its molecular mechanisms.

To delineate the anti-inflammatory effect of ginsenosides, we first examined tumor necrosis factor-α (TNF-α)-induced

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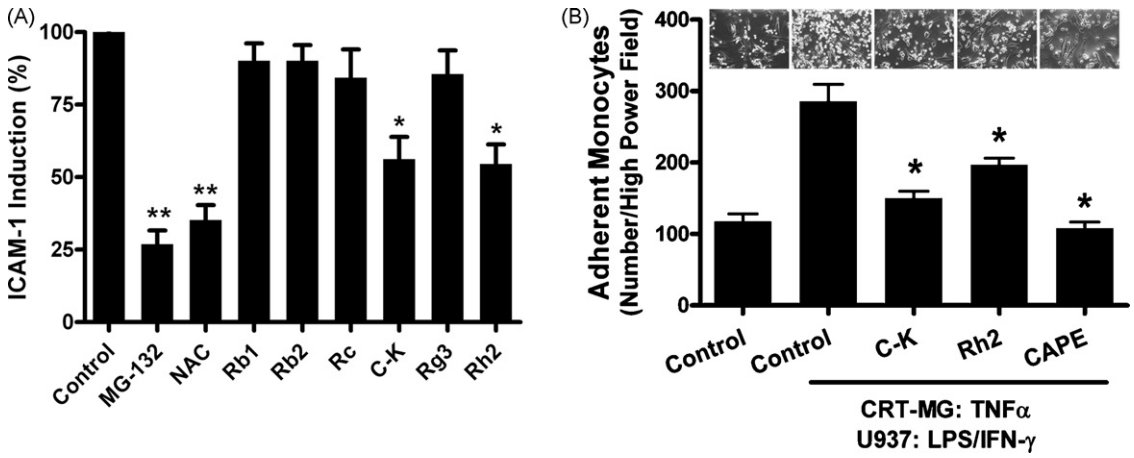

Fig. 1. Inhibitory effect of ginsenoside C-K and Rh_2 on TNF-α-induced ICAM-1 expression in human astroglial cells. (A) CRT-MG cells were incubated in the absence and presence of various ginsenosides (20 μg/ml) for 1 h, treated with TNF-α (10 ng/ml) for an additional 24 h, and then ICAM-1 protein expression level was measured by flow cytometric analysis. Data indicate the mean fluorescence intensity (mean ± S.D.) of quadruplicate samples. Asterisk indicates samples statistically significantly different from the control group treated with TNF-α alone (Tukey post-hoc test applied to significant effect of group ANOVA $F_{8,27} = 16.39$, $P < 0.001$), (*) $P < 0.005$, (**) $P < 0.001$. Representative of three independent experiments. (B) CRT-MG cells were incubated in the absence or presence of ginsenosides (20 μg/ml) or CAPE (30 μg/ml) for 1 h, treated with TNF-α for an additional 24 h, and cocultured with activated U937 cells for 4 h. Adherent monocytes were counted after nonadherent cells were removed by gentle wash steps. Asterisk indicates the statistics of samples, which is significantly different from the control group, which was treated with TNF-α alone (Tukey post-hoc test applied to significant effect of group ANOVA $F_{4,45} = 27.50$, $P < 0.001$), $P < 0.001$. Representative of five independent experiments.

expression of ICAM-1 as a read-out for astroglial activation. ICAM-1 is a single-chain 76–114-kDa glycoprotein and binds to LFA-1 (CD11a/CD18), Mac-1 (CD11b/CH18), hyaluronate, and CD43. Reactive astrocytes and microglia express ICAM-1, which increases upon stimulation with TNF-α, interleukin-1β, and interferon-γ [7,14]. The major functions of ICAM-1 are endothelial–leukocyte cell interactions, leukocyte extravasation, and antigen presentation as an accessory molecule. Ginsenosides were first tested for their inhibitory effect on TNF-α-induced ICAM-1 protein expression by astroglial cells using FACS analysis (Fig. 1). Naturally occurring ginsenosides and ginsenoside metabolites (compound K (C-K), Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_2, Ro, and F_1) were generously provided from the Central Research Institute of KT&G (Daejeon, Korea). *N*-acetylcysteine and MG-132 were obtained from Calbiochem (San Diego, CA, USA). The CRT-MG human astroglioma cells were maintained in RPMI 1640 medium with 10 mM HEPES (pH 7.2) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco BRL, Grand Island, NY, USA), and 10% heat-inactivated FBS as previously described [5]. Primary human fetal astrocytes were isolated and maintained, as described previously [6]. For flow cytometric analysis, cells (2×10^5 cells/well) were plated in six-well (35-mm^2) plates (Costar, Cambridge, MA, USA) and grown to 90% confluency. Cells were incubated in the absence or presence of ginsenosides for 1 h then treated with 10 ng/ml of TNF-α (R&D Systems, Minneapolis, MN, USA) for an additional 24 h. For analysis of intercellular adhesion molecule-1 (ICAM-1) expression, cells were stained with mouse anti-ICAM-1 antibody conjugated with phycoerythrin (1:500) (Serotec, Washington, DC, USA), as previously described [7]. Negative controls were incubated with a mouse isotype-matched (IgG_1) antibody conjugated with phycoerythrin (1:100) (Southern Biotechnology Associates, Birmingham, AL, USA). Ten thousand cells were analyzed for each sample. Statistical analyses of ICAM-1 expression were performed by ANOVA with Tukey's honest significant difference post-hoc test applied to significant main effects or interactions (SPSS 12.0K for Windows, SPSS, Chicago, IL, USA).

TNF-α-induced ICAM-1 expression of astroglial cells was significantly attenuated by a proteasomal inhibitor MG-132 [17] and a reactive oxygen species (ROS) scavenger *N*-acetylcystein, suggesting the involvement of the NF-κB and ROS in TNF-α-induced signals. Interestingly, intestinal bacterial metabolites of ginsenosides, including C-K and Rh_2 [1], showed a significant inhibitory effect on TNF-α-induced ICAM-1 protein expression by astroglial cells. The inhibitory effect of C-K and Rh_2 was still observed at a concentration as low as 5 μg/ml of ginsenoside, while other ginsenosides had no effect at a concentration up to 40 μg/ml (data not shown). The inhibitory effect of C-K and Rh_2 on ICAM-1 expression was also observed in the astroglial cells treated with interleukin-1β (data not shown). We next examined the effect of ginsenosides on ICAM-1-mediated monocyte adhesion (Fig. 1B). U937 cells were used for the adhesion assay, as previously described [19]. U937 cells were incubated in the presence of lipopolysaccharides (LPS, 100 ng/ml) and interferon-γ (100 U/ml) for 24 h and incubated with adherent astroglial cells for 4 h, and non-adherent U937 cells were removed by gentle wash steps. Treatment of astroglial cells with TNF-α markedly increased the monocytic adhesion. Pretreatment of astroglial cells with C-K or Rh_2 significantly suppressed TNF-α-induced monocytic adhesion, which was consistent with ICAM-1 expression results. Treatment with caffeic acid phenethyl ester (CAPE), a well-known anti-inflammatory propolis [15], also suppressed TNF-α-induced monocytic adhesion. Since ginsenoside C-K and Rh_2 exerted