ORIGINAL ARTICLES

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Ginsenoside Rg3 inhibits lipopolysaccharide–induced adhesion molecule expression in human umbilical vein endothelial cell and C57BL/6 mice

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Intercellular adhesion molecule 1 (ICAM–1), vascular cell adhesion molecule 1 (VCAM–1), P– and E–selectin play a key role for initiation of vascular inflammation. Ginsenoside, a class of steroid glycosides, is abundant in *Panax ginseng* root, which has been used for health promotion in Korea. In this study, we investigated the mechanism by which ginsenoside Rg3 may inhibit ICAM–1 and VCAM–1 expressions stimulated with lipopolysaccharide (LPS) in human umbilical vein endothelial cell (HUVEC) and C57BL/6 mice. LPS increased ICAM–1 and VCAM–1 expression. Ginsenoside Rg3 prevented LPS–mediated increase of ICAM–1 and VCAM–1 expression. LPS induced IkappaBα (IκBα) degradation within 1 hr. Ginsenoside Rg3 prevented the IκBα degradation stimulated with LPS. Moreover, ginsenoside Rg3 reduced LPS–mediated THP–1 monocyte adhesion to HUVEC, in a concentration–dependent manner. In C57BL/6 mice, injection of LPS increased aortic ICAM–1 and VCAM–1 expression, which was prevented by ginsenoside Rg3. These data provide a novel mechanism where the ginsenoside Rg3 may provide direct vascular benefits with inhibition of leukocyte adhesion into vascular wall thereby providing prevention against vascular inflammatory disease.

1. Introduction

Activation of vascular endothelial cells is the initiating event of vascular inflammation. Adhesion molecules initiate leukocyte–endothelial cell contact and facilitate subsequent rolling, activation and transendothelial migration into vascular wall. Recruited monocytes differentiate into macrophages, which aggravates vascular inflammation (Kuldo et al. 2005; Libby 2007).

On the other hand, gram–negative bacterial endotoxin lipopolysaccharide (LPS) provokes a low–grade inflammation. Low–grade endotoxemia may cause a mild pro–inflammatory state, which eventually leads to the pathogenesis and progression of inflammatory diseases (Glaros et al. 2013). In human umbilical vein endothelial cells (HUVEC), LPS increased the expression of endothelial–leukocyte adhesion molecule–1 (E–selectin), intercellular adhesion molecule 1 (ICAM–1) and vascular cell adhesion molecule 1 (VCAM–1) (Rao et al. 2013; Jiang et al. 2013). Moreover, E. coli LPS–induced endotoxemia resulted in an increase in circulating plasma markers of endothelial activation ICAM–1 and VCAM–1 in human (Ramakers et al. 2011).

Ginsenosides, found in the plant genus *Panax*, are triterpene saponins. These can be classified into two groups as follows: the protopanaxadiol group (Rb1, Rb2, Rc, Rd, and Rg3) and the protopanaxatriol group (Rg1, Re, Rf, Rg2). Korean ginseng berry extract administration suppressed NF–kappaB–mediated expression ICAM–1 and VCAM–1 without altering serum cholesterol levels, in ApoE(–/–) mice fed a high fat–diet (Kim et al. 2012). Moreover, Ginsenoside Rg2 prevented an increase of VCAM–1 and ICAM–1 expression induced by LPS in HUVEC (Cho et al. 2013).

Therefore, ginsenoside may act as a potential molecule for prevention and treatment against vascular inflammation. However, the effect of ginsenoside on decrement of vascular inflammation has not been fully investigated *in vivo*. The aim of study was to investigate the effect and mechanism of ginsenoside Rg3 on LPS–induced ICAM–1 and VCAM–1 expression in HUVEC and C57BL/6 mice.

2. Investigations and results

2.1. Effect of ginsenoside Rg3 on LPS–induced ICAM–1 and VCAM–1 expression in endothelial cells

Treatment of endothelial cells with LPS (1 μg/ml) increased ICAM–1 and VCAM–1 expression. Eight hours after LPS treatment, maximal expression of ICAM–1 and VCAM–1 was attained (data not shown). To determine whether LPS–stimulated ICAM–1 and VCAM–1 expression is affected by ginsenoside Rg3, endothelial cells were treated for 1 h with ginsenoside Rg3 (1–50 μM) prior to LPS (1 μg/ml) stimulation for 8 h. Ginsenoside Rg3 significantly inhibited ICAM–1 and VCAM–1 expression stimulated with LPS in a concentration–dependent manner (Fig. 1).

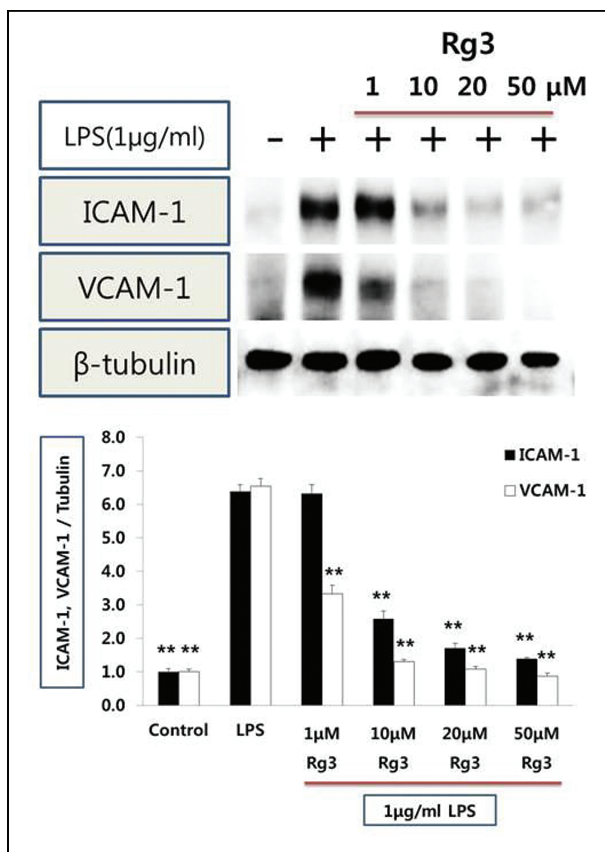

Fig. 1: Effect of ginsenoside Rg3 on the protein expression levels of adhesion molecules in HUVEC stimulated with LPS. Endothelial cell was treated with 1, 10, 20 and 50 μM of ginsenoside Rg3 for 1 h prior to LPS (1 μg/ml) stimulation for 8 h. Cell extracts were resolved on 8% SDS–polyacrylamide gel and Western blot analysis with the respective primary antibody against ICAM–1 and VCAM–1. β–tubulin was used as an internal control. The bar graph represents the amount of ICAM–1 and VCAM–1 estimated by image scanning and is expressed in arbitrary units. Values are means ± SEM of 3 independent experiments. Statistical significance assessed by one—way ANOVA followed by Scheffe *post–hoc* test for multiple comparisons (**$P < 0.01$ vs LPS).

2.2. *Effect of ginsenoside Rg3 on LPS-induced IκBα degradation in endothelial cells*

Treatment of endothelial cells with LPS (1 μg/ml) increased IκBα degradation. One hour after LPS treatment, a significant decrease of IκBα was attained (Fig. 2A). To determine whether LPS–induced IκBα degradation was affected by ginsenoside Rg3, endothelial cells were treated for 1 h with ginsenoside Rg3 (1–50 μM) prior to LPS (1 μg/ml) stimulation for 1 h. Ginsenoside Rg3, significantly prevented LPS–induced IκBα degradation in a concentration–dependent manner (Fig. 2B).

2.3. *Effect of ginsenoside Rg3 on THP–1 cell adhesion in endothelial cells*

The adhesion of THP–1 cells to endothelial cells was measured using quantitative monolayer adhesion assay. The adhesion of THP–1 cells onto endothelial cells were increased to five folds by LPS (1 μg/ml) stimulation for 8 h. Ginsenoside Rg3 (1–50 μM) inhibited the adhesion of THP–1 cells to endothelial cells stimulated with LPS, in a concentration-dependent manner (Fig. 3). Moreover, JSH (50 μM), inhibitor of NF–κB, significantly attenuated the adhesion of THP–1 cells to endothelial cells stimulated with LPS (Fig. 3).

2.4. *Effect of ginsenoside Rg3 on LPS-induced adhesion molecules expression in C57BL/6 mice*

Injection of LPS (20 mg/kg) into abdominal cavity of C57BL/6 mice increased ICAM–1 and VCAM–1 expression onto abdominal vascular endothelium (Fig. 4). Pretreatment of Rg3 (20 mg/kg) abolished ICAM–1 and VCAM–1 expression induced by LPS onto abdominal vascular endothelium in C57BL/6 mice (Fig. 4).

3. Discussion

Ginsenoside, a class of steroid glycosides, is abundant in *Panax ginseng* root, which has been used for promotion of health in Korea. Ginsenoside Rg3, especially in combination with chemotherapy, might be correlated with improving the immune function and anti–tumor angiogenesis in human (Lu et al. 2008). Ginsenoside Rg1 had a protective role against cecal ligation and puncture–induced polymicrobial sepsis by attenuating the proinflammatory response, enhancing innate immunity and preserving adaptive immunity (Zou et al. 2013). Ginsenoside Rg3 inhibited tumor necrosis factor-alpha (TNFα)–induced protein and mRNA expression of ICAM–1 and VCAM–1 in human endothelial cells (Hien et al. 2010). However, the inhibitory effect of ginsenoside Rg3 on expression of ICAM–1 and VCAM–1 in vascular endothelial cell was not proved *in vivo* study.

In this study, ginsenoside Rg3 was also effective for suppression of an expression of ICAM–1 and VCAM–1 stimulated with LPS in HUVEC. Moreover, in C57BL/6 mice, ginsenoside Rg3 was effective for suppression of vascular expression of ICAM–1 and VCAM–1 induced by LPS. This is the first report of effect of ginsenoside Rg3 on expression of ICAM–1 and VCAM–1 by LPS *in vivo*. Ginsenoside Rg3 prevented the IκBα degradation stimulated with LPS. This result is consistent with a previous report of ginsenoside Rg2 or ginseng berry extract (Kim et al. 2012; Cho et al. 2013). IκB dissociation from RelA–p50 complex is crucial for NF–κB activity (Monaco and Paleolog 2004). Therefore, ginsenoside Rg3 seems to down-regulate NF–κB activity, which results in suppression of ICAM–1 and VCAM–1 in vascular endothelium.

On the other hand, LPS, the prototypical endotoxin, promotes the secretion of pro–inflammatory cytokines in many cell types after binding onto CD14/TRL4/MD2 receptor (Rockel and Hartung 2012). Downstream signaling of the TLR4 receptor complex in response to LPS is largely mediated $p38^{MAPK}$/MK2 pathway (Bode et al. 2012). Pro–inflammatory cytokines such as TNFα, interleukin–1β (IL–1β) and interleukin–6 (IL–6) are expressed by activation of $p38^{MAPK}$ signaling pathway. $P38^{MAPK}$ inhibitor decreased the expression of ICAM–1 stimulated with vascular endothelial growth factor (VEGF) in retinal endothelial cell (Zhang et al. 2009). Moreover, $P38^{MAPK}$ inhibitor decreased the expression of VCAM–1 stimulated with TNFα in human proximal tubular epithelial cell (Ho et al. 2008).

LPS–mediated inflammatory responses involve the transcriptional activity of activator protein (AP)–1 and/or interferon regulatory factor (IRF)–3 in addition to NF–κB (Kim et al. 2011). Endothelial MAPK kinase 3 (MKK3), the $p38^{MAPK}$ group, was required for inflammatory cell recruitment to the lungs, mitochondrial oxidant-mediated AP–1, NF–κB activation, and ICAM–1 expression during LPS challenge (Mannam et al. 2013). On the other hand, LPS activates IRF–3 in a $p38^{MAPK}$–dependent manner. IRF–3 participates in the transcriptional induction of interferon (IFN)–alpha, IFN–beta, and a subset of IFN–stimulated genes (ISGs) as a result of viral infection (Chiang et al. 2006). Therefore, there is the possibility that

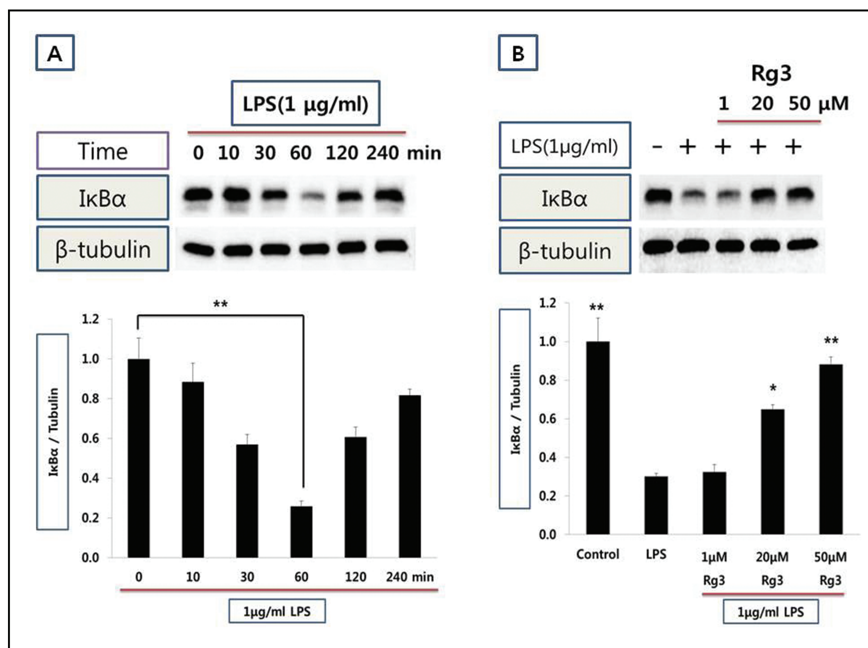

Fig. 2: Effect of ginsenoside Rg3 on IκBα degradation in HUVEC stimulated with LPS. (A) Time–course of IκBα degradation in HUVECs stimulated with LPS (1 μg/ml). (B) Effect of ginsenoside Rg3 on time–course of IκBα degradation in HUVEC stimulated with LPS. Endothelial cell was treated with 1, 20 and 50 μM of ginsenoside Rg3 prior to LPS (1 μg/ml) stimulation for 1 h. Cell extracts were resolved on 10% SDS–polyacrylamide gel and Western blot analysis with the respective primary antibody against IκBα. β–tubulin was used as an internal control. The bar graph represents the amount of IκBα estimated by image scanning and is expressed in arbitrary units. Values are means ± SEM of 3 independent experiments. Statistical significance assessed by one—way ANOVA followed by Scheffe *post–hoc* test for multiple comparisons (A $^{**}P < 0.01$ vs control; B $^{*}P < 0.05$, $^{**}P < 0.01$ vs LPS).

ginsenoside Rg3 might regulate the $p38^{MAPK}$ signaling pathway stimulated with LPS resulting in inhibition of the transcriptional activity of AP–1 and/or IRF–3 in addition to NF–κB in vascular endothelial cell although further study remains.

The increase of THP–1 monocyte adhesion to endothelial cells by LPS supports the crucial role of ICAM–1 and VCAM–1 in the early phase of vascular inflammation. It was reported that TNFα–induced THP–1 monocyte adhesion to HUVEC was attenuated by ginsenoside Rh1 (Lee et al. 2011). Ginsenoside Rg2 also attenuated THP–1 monocyte adhesion to HUVEC stimulated with LPS (Cho et al. 2013). In this study, we showed that ginsenoside Rg3 significantly reduced THP–1 monocyte adhesion to vascular endothelial cells stimulated with LPS in a concentration–dependent manner. Therefore, ginsensoside Rg3 may be effective against early phase of vascular inflammation.

In conclusion, ginsenoside Rg3 may provide direct vascular benefits with inhibition of leukocyte adhesion into vascular wall thereby providing prevention against vascular inflammatory disease.

4. Experimental

4.1. Reagents

Endothelial cell basal medium (EBM)–2 Bullet kit was obtained from Lonza (USA). RPMI 1640, fetal bovine serum (FBS), penicillin–streptomycin, phosphate buffer saline (PBS), trypsin–EDTA were obtained from *Invitrogen* (USA). Lipopolysaccharide (LPS) was obtained from List Biological Laboratories (USA). Gelatin, Calcein–AM, JSH and ginsenoside Rg3 were obtained from Sigma–Aldrich (USA). Antibodies against human vascular cell adhesion molecule-1 (VCAM–1), human intercellular adhesion molecule-1 (ICAM–1), IκBα, β–tubulin and HRP–conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (USA).

4.2. Cell culture

Human umbilical vein endothelial cells (HUVECs) were purchased from Lonza (USA) at passage 1 and maintained in EBM–2 Bullet kit growth medium at 37 °C in a humidified atmosphere of 5% CO_2. In all experiments, cells were used at passage 4∼9. HUVECs were plated at 90∼95% confluence treated for 1 h with 1, 10, 20 and 50 μM of Rg3 prior to LPS (1 μg/mL) stimulation for 1 or 8 h. A group of cells treated only with dimethylsulfoxide (DMSO) were used as a solvent control. Human-derived THP-1 macrophage cell lines were obtained from the American Type Culture Collection (ATCC) (USA). THP-1 cells were cultured in RPMI 1640, and supplemented with 2 mM L–lutamine, 100 μg/ml streptomycin, 100 IU/ml penicillin and 10% FBS.

4.3. Western blotting

The endothelial cells were pretreated with Rg3 prior to LPS stimulation. After treatment, the cells were washed twice in PBS. Whole cell lysates were prepared RIPA buffer containing the Protease Inhibitor Cocktail V (Switzerland). Protein concentration was determined using the Bio-Rad protein assay (Bio–Rad Lab, USA) with BSA as the standard. The lysates were resolved on 8% or 10% SDS–polyacrylamide gel. The proteins were electrophoretically transferred to an Immobilon–P membrane (Millipore, USA) and the membranes were blocked with 5% nonfat dry milk in Tris–buffered saline containing 0.1% Tween–20 (TTBS) at room temperature for 1 h. The membrane was incubated overnight with primary antibodies of anti–human ICAM–1, anti–human VCAM–1 and IκBα. After three washes with TTBS buffer, the membrane was incubated for 1 h with HRP–conjugated secondary antibodies. The levels of ICAM–1, VCAM–1 and IκBα proteins were determined using an Enhanced Chemoluminescence Plus kit (Amersham Biosciences, USA) and Fujifilm LAS–3000 system (Fujilfilm, Japan). Anti–human β–tubulin antibody was used for the loading control. Each image of Western blot was quantified with Multi Gauge software version 2.3 (Fujifilm, Japan).

4.4. Cell adhesion assay

HUVECs were grown in EBM–2 Bullet kit growth medium at a density of 2.0×10^5 cells/well on 24–well plates. Endothelial cells at 90–95% confluence were treatment with Rg3 (1, 20 and 50 μM) and JSH 50 μM for 1 h prior to 1 μg/ml of LPS stimulation for 8 h. THP–1 cells were labeled with Calcein–AM (5 μM) in RPMI 1640 medium containing 10% FBS for 30 min. After two times of extensive washing with PBS, the labeled THP-1 cells were seeded at a density of 5.0×10^5 cells/well onto endothelial cells which were treated with the Rg3, JSH and/or LPS and, then incubated for 1 h at 37 °C while gentle shaking. After incubation, non–adherent cells were removed by gentle washing two times with

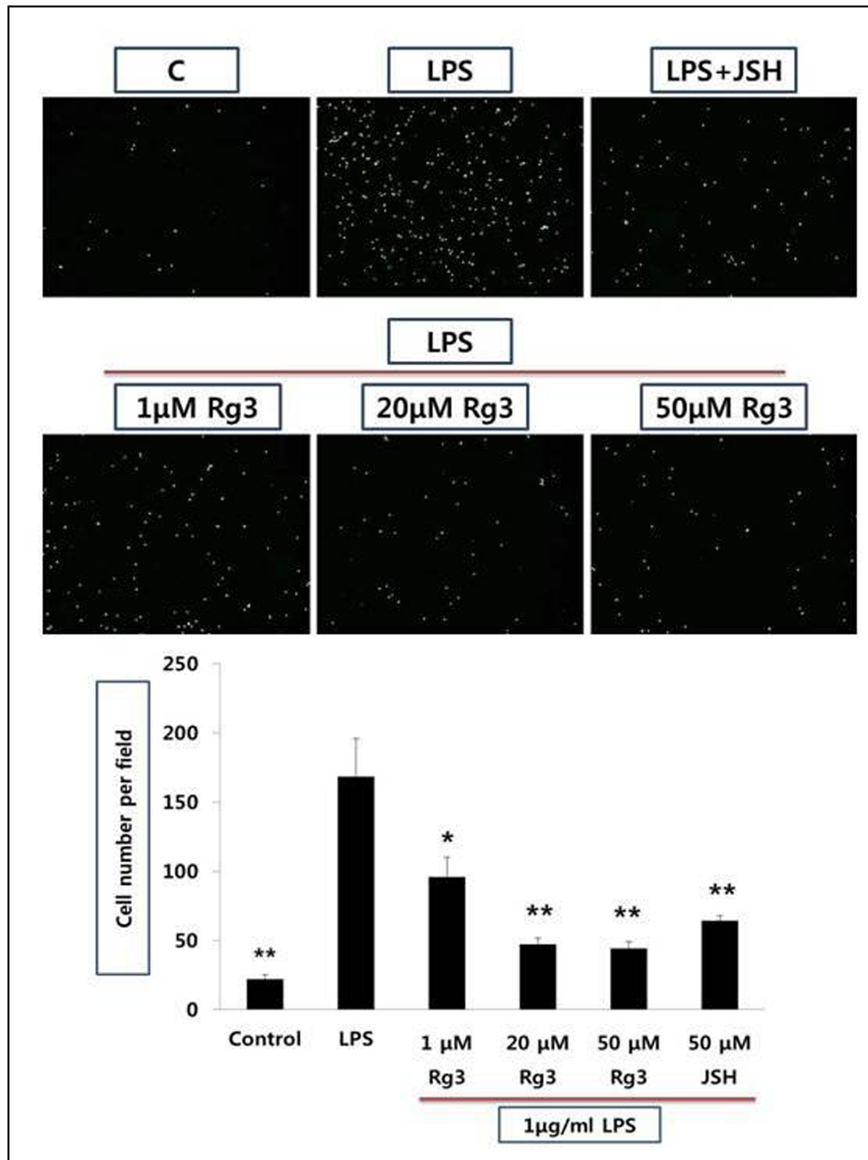

Fig. 3: Effect of ginsenoside Rg3 on THP–1 monocyte adhesion to LPS in HUVEC stimulated with LPS. (A) Endothelial cell was treated with 1, 20 and 50 μM of ginsenoside Rg3 prior to LPS (1 μg/ml) stimulation for 8 h. THP–1 cells were labeled with Calcein–AM (5 μM) for 30 min. The labeled THP–1 cells were seeded at a density of 5.0 × 100,000 cells/well onto endothelial cells treated with the ginsenoside Rg3 and/or LPS and then incubated for 1 h. Microphotographs (four independent experiments) were obtained using fluorescence microscopy. Magnification × 100 (B) The bar graph represents the cell number of THP–1 monocyte. Values are means ± SEM of 3 independent experiments. Statistical significance assessed by one–way ANOVA followed by Scheffe *post–hoc* test for multiple comparisons (*$P < 0.05$, **$P < 0.01$ vs LPS).

PBS. Photograph images were obtained at 485 nm excitation and 538 nm emission using a SPOT II digital camera–attached fluorescence microscope.

4.5. Animal study

Male C57BL/6 mice, 8 weeks of age (body weight 18–22 g, Sam Tako, Osan, Korea) have been used. Mice were randomly distributed into three groups: S saline (group 1, n = 5), LPS 20 mg/kg (group 2, n = 5) and LPS 20 mg/kg + Rg3 20 mg/kg (group 3, n = 5). Rg3 was administered 1 h before LPS injection. Eight hours later, mice were anesthetized with ketamine (30 mg/kg) and xylazine (6 mg/kg) and their abdominal aorta was collected for immunohistochemistry. The experimental protocol was approved by Chungbuk National University Medical School Research Institutional Animal Care and Use Committee.

4.6. Immunohistochemistry

ICAM–1 and VCAM–1 were analyzed by immunohistochemistry staining in formalin-fixed, paraffin-embedded biopsy sections of mice abdominal aorta. Serial sections (4 μm) were cut from each paraffin block, and immunostained by deparaffinizing and rehydrating through a graded alcohol series. Antigens were retrieved by heating the sections in a microwave for 5 min in 10 mM sodium citrate (pH 6.0). After blocking nonspecific sites, sections were incubated overnight at 4 °C with anti–ICAM–1 (1:100) or anti–VCAM–1 (1:100) antibodies. To visualize, sections were stained using a standard method avidin–biotin–horseradish peroxidase complex was used to localize bound antibody, and diaminobenzidine was used as the final chromogen. For histological assessment, ICAM–1 and VCAM–1 were identified at magnifications of 400 ×.

4.7. Statistical analysis

The results are presented as means ± SEM for each treatment group in each experiment. Data were tested by one–way ANOVA followed by Scheffe *post–hoc* test for multiple comparisons. Statistical software SPSS (USA) was used in. P values < 0.05 were considered statistically significant.

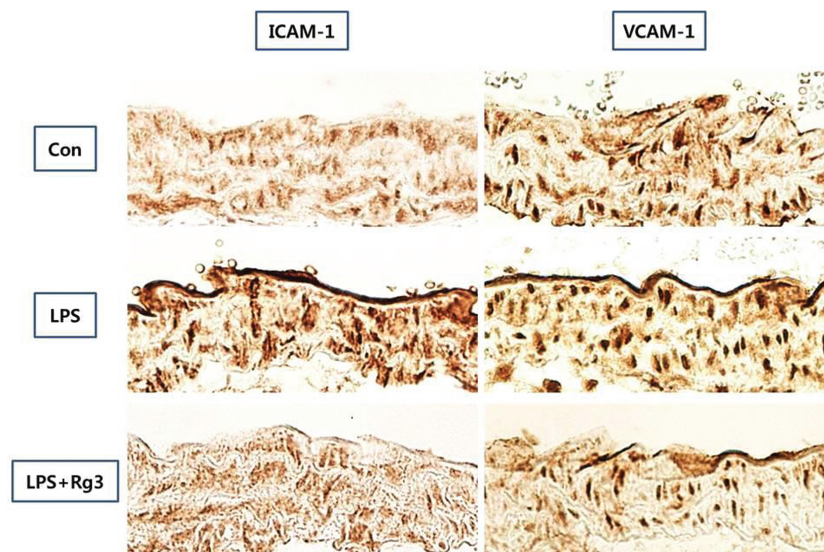

Fig. 4: Effect of ginsenoside Rg3 on the protein expression levels of adhesion molecules in the C57BL/6 mice aorta stimulated with LPS. Mice were randomly distributed into three groups: Saline (group 1, n = 5), LPS 20 mg/kg (group 2, n = 5) and LPS 20 mg/kg + ginsenoside Rg3 20 mg/kg (group 3, n = 5). Ginsenoside Rg3 was administered 1 h before LPS injection. After 8 h later, mice was anesthetized with ketamine (30 mg/kg) and zylazine (6 mg/kg) and abdominal aorta was collected for immunohistochemistry. ICAM–1 and VCAM–1 were analyzed by staining in formalin–fixed, paraffin–embedded biopsy sections of mice abdominal aorta. For histological assessment, ICAM–1 and VCAM–1 were indentified at magnifications of × 400.

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References

Bode JG, Ehlting C, Haussinger D (2012) The macrophage response towards LPS and its control through the p38(MAPK)-STAT3 axis. Cell Signal 24: 1185–1194.

Chiang E, Dang O, Anderson K, Matsuzawa A, Ichijo H, David M (2006) Cutting edge: apoptosis-regulating signal kinase 1 is required for reactive oxygen species-mediated activation of IFN regulatory factor 3 by lipopolysaccharide. J Immunol 176: 5720–5724.

Cho YS, Kim CH, Ha TS, Lee SJ, Ahn HY (2013) Ginsenoside Rg2 inhibits lipopolysaccharide-induced adhesion molecule expression in human umbilical vein endothelial cell. Kor J Physiol Pharmacol 17: 133–137.

Glaros TG, Chang S, Gilliam EA, Maitra U, Deng H, Li L (2013) Causes and consequences of low grade endotoxemia and inflammatory diseases. Front Biosci 5: 754–765.

Hien TT, Kim ND, Kim HS, Kang KW (2010) Ginsenoside Rg3 inhibits tumor necrosis factor-alpha-induced expression of cell adhesion molecules in human endothelial cells. Pharmazie 65: 699–701.

Ho AW, Wong CK, Lam CW (2008) Tumor necrosis factor–alpha up–regulates the expression of CCL2 and adhesion molecules of human proximal tubular epithelial cells through MAPK signaling pathways. Immunobiology 213: 533–544.

Jiang SJ, Hsu SY, Deng CR, Huang HC, Liu SL, Shi GY, Wu HL (2013) Dextromethorphan attenuates LPS–induced adhesion molecule expression in human endothelial cells. Microcirculation 20: 190–201.

Kim CK, Cho DH, Lee KS, Lee DK, Park CW, Kim WG, Lee SJ, Ha KS, Goo TO, Kwon YG, Kim YM (2012) Ginseng berry extract prevents atherogenesis via anti–inflammatory action by upregulating phase II gene expression. Evid Based Complement Alternat Med 2012: 490301.

Kim MH, Yoo DS, Lee SY, Byeon SE, Lee YG, Min T, Rho HS, Rhee MH, Lee J, Cho JY (2011) The TRIF/TBK1/IRF-3 activation pathway is the primary inhibitory target of resveratrol, contributing to its broad-spectrum anti–inflammatory effects. Pharmazie 66: 293–300.

Kuldo JM, Ogawara KI, Werner N, Asgeirsdottir SA, Kamps JA, Kok RJ, Molema G (2005) Molecular pathways of endothelial cell activation for (targeted) pharmacological intervention of chronic inflammatory diseases. Curr Vasc Pharmacol 3: 11–39.

Lee ES, Choi JS, Kim MS, You HJ, Ji GE, KangYH (2011) Ginsenoside metabolite compound K differentially antagonizing tumor necrosis factor–alpha–induced monocyte–endothelial trafficking. Chem Biol Interact 194: 13–22.

Libby P (2007) The molecular basis of inflammation and disease. Nutr Rev 65: S140–146.

Lu P, Su W, Miao ZH, Niu HR, Liu J, Hua QL (2008) Effect of mechanism of ginsenoside Rg3 on postoperative life span of patients with non–small cell lung cancer. Chin J Integr Med 14: 33– 36.

Mannam P, Zhang X, Shan P, Zhang Y, Shinn AS, Zhang Y, Lee PJ (2013) Endothelial MKK3 is a critical mediator of lethal murine endotoxemia and acute lung injury. J Immunol 190: 1264–1275.

Monaco C, Paleolog E (2004) Nuclear factor kappaB: a potential therapeutic target in atherosclerosis and thrombosis. Cardiovasc Res 61: 671–682.

Ramakers BP, Riksen NP, van den Broek P, Franke B, Peters WH, van der Hoeven JG, Smits P, Pickkers P (2011) Circulating adenosine increases during human experimental endotoxemia but blockade of its receptor does not influence the immune response and subsequent organ injury. Crit Care 15: R3.

Rao TO, Okamoto T, Akita N, Hayashi T, Kato–Yasuda N, Suzuki K (2013) Amla (Emblica officinalis Gaertn.) extract inhibits lipopolysaccharide–induced procoagulant and pro–inflammatory factors in cultured vascular endothelial cells. Br J Nutr 110: 2201–2206.

Rockel C, Hartung T (2012) Systemic review of membrane components of gram–positive bacteria responsible as pyrogens for inducing human monocyte/macrophage cytokine release. Front Pharmacol 3: 56.

Zhang XL, Wen L, Chen YJ, Zhu Y (2009) Vascular endothelial growth factor up-regulates the expression of intracellular adhesion molecule–1 in retinal endothelial cells via reactive oxygen species, but not nitric oxide. Chin Med J 122: 338–343.

Zou Y, Tao T, Tian Y, Zhu J, Cao L, Deng X, Li J (2013) Ginsenoside Rg1 improves survival in a murine model of polymicrobial sepsis by suppressing the inflammatory response and apoptosis of lymphocytes. J Surg Res 183: 760–766.