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Preventive effect of 20(*S*)-ginsenoside Rg_3 against lipopolysaccharide-induced hepatic and renal injury in rats

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Abstract

The preventive effect of 20(*S*)-ginsenoside Rg_3 (20(*S*)-Rg_3) on lipopolysaccharide (LPS)-induced oxidative tissue injury in rats was investigated in this study. The elevated serum nitrite/nitrate, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and creatinine levels in LPS-treated control rats were significantly decreased following 15 consecutive days of 20(*S*)-Rg_3 administration. In addition, thiobarbituric acid-reactive substance levels in the serum, liver and kidney were dose-dependently lower in 20(*S*)-Rg_3-treated groups than in the LPS-treated control group. The nuclear factor-κB (NF-κB), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and heme oxygenase-1 (HO-1) protein expressions in the liver and kidney were significantly increased by LPS treatment. However, the 20(*S*)-Rg_3 administrations significantly decreased these protein expressions except for HO-1 in the liver. On the other hand, in the kidney, oral administration of 20(*S*)-Rg_3 showed a tendency to reduce NF-κB and iNOS protein expressions and also significantly reduced the elevated COX-2 and HO-1 protein expressions at a dose of 10 mg/kg body weight/day. All these results suggest the preventive effect of 20(*S*)-Rg_3 against LPS-induced acute oxidative damage in the liver and kidney and the preventive effect of 20(*S*)-Rg_3 administration against LPS toxicity was thought to be more predominant in the liver than kidney.

Keywords: *20(S)-Ginsenoside Rg_3, lipopolysaccharide, antioxidant, anti-inflammatory*

Introduction

Panax ginseng C. A. MEYER (Araliaceae) is one of the most widely used herbal medicines in the Orient. It has been used for many years as an anti-ageing and anti-mutagenic agent and for the treatment of neuronal disorder and cardiovascular diseases [1–4]. These human diseases involve tissue damage that will inevitably be accompanied by local oxidative stress, which exacerbates the damage [5]. Therefore, the various pharmacological efficacies of *Panax ginseng* were thought to be mediated, not totally, by its protective effects against free radical attack.

Ginseng extract was reported to scavenge hydroxyl radicals ($^{\bullet}OH$), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, superoxide anions ($O_2^{\bullet -}$) and peroxynitrite ($ONOO^-$) [6–8]. In addition, significant increases in the nitric oxide ($^{\bullet}NO$)-, $O_2^{\bullet -}$-,$^{\bullet}OH$- and $ONOO^-$-scavenging activities of *Panax ginseng* brought about by heat processing were confirmed by our previous research [9–11]. Moreover, lipopolysaccharide (LPS)- and streptozotocin-induced hepatic and renal oxidative damage, respectively, were significantly ameliorated by the administration of heat-processed *Panax ginseng* extract [12,13]. On the other hand, there is considerable interest in the isolation of more

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potent antioxidant compounds to treat diseases involving oxidative stress from herbal medicines [14] and we have been investigating the main antioxidant compound of heat-processed *Panax ginseng* by activity-guided fractionation and component analysis.

Ginsenosides, a diverse group of steroidal saponins that target a vast range of tissues, and their metabolites, are generally believed to be mainly responsible for the pharmacological activity of ginseng [15,16]. Among the several ginsenosides, 20(*S*)-ginsenoside Rg_3 (20(*S*)-Rg_3, Figure 1) was screened out as the strongest $^{\bullet}OH$-scavenging compound of heat-processed *Panax ginseng* by the ferrous metal ion chelating activity and its content was significantly increased by heat processing [11,17]. Although the potential antioxidant and anti-inflammatory effects of 20(*S*)-Rg_3 have been examined [18–20], there are virtually no reports comparing the effect of orally administered 20(*S*)-Rg_3 on oxidative damage in the liver and kidney, the major organs impaired by oxidative stress [21]. This comparison of the effects in different organs was thought to be useful to identify the tissue-specific antioxidant mechanisms of 20(*S*)-Rg_3.

On the other hand, it has been well-defined that reactive oxygen species (ROS) are involved in the mechanism of LPS toxicity, in particular nuclear factor-κB (NF-κB) activation, and metal chelators such as deferoxamine are known to reduce tissue injury and lethality in LPS-treated mice [21–23]. Therefore, the preventive effect of 20(*S*)-Rg_3 against LPS-induced hepatic and renal injury in rats was compared in this study.

Materials and methods

Materials

LPS (from *Escherichia coli* serotype 055: B5), phenylmethylsulfonyl fluoride (PMSF) and β-actin were purchased from Sigma Chemical Co. (St. Louis, MO). NF-κBp65, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), heme oxygenase-1 (HO-1) and goat anti-rabbit and/or goat anti-mouse IgG horseradish peroxidase (HRP) conjugated secondary antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). 20(*S*)-Rg_3 was previously isolated from *Panax ginseng* by the reported methods [24]. The other chemicals and reagents used were of high quality and obtained from commercial sources.

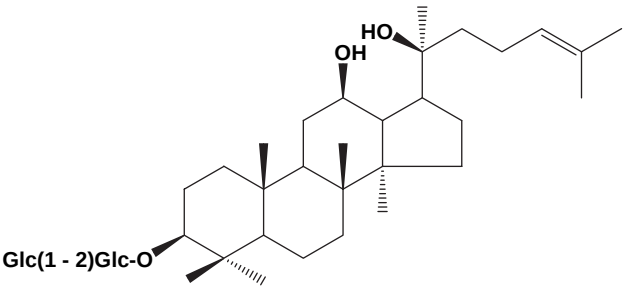

Figure 1. The chemical structure of 20(*S*)-Rg_3. –Glc: D-glucopyranosyl.

Animal experiment

The Guidelines for Animal Experimentation, approved by the University of Toyama, were followed in these experiments. Male Wistar rats (120–130 g) from Japan SLC, Inc. (Hamamatsu, Japan) were used. They were allowed free access to laboratory pellet chow (CLEA Japan Inc., Tokyo, Japan) and water. After several days of adaptation, the animals were divided into four groups (each group consisted of six rats), avoiding any inter-group differences in body weight. The control group was given water, while the other groups were orally administered 20(*S*)-Rg_3 at a dose of 5 or 10 mg/kg body weight daily using a stomach tube. After 15 consecutive days of administration, the rats were given intraperitoneal LPS, 5 mg/kg body weight. At 6 h after LPS challenge, blood samples were collected from the abdominal aorta. The serum was immediately separated from the blood samples by centrifugation. Subsequently, the liver and kidney were perfused through the artery with ice-cold physiological saline (0.9% NaCl, pH 7.4), removed, quickly frozen and kept at $-80^{\circ}C$ until analysis.

Assays of serum parameters

The nitrite/nitrate (NO_2^-/NO_3^-) level was determined by converting NO_3^- to NO_2^- using the enzyme nitrate reductase, followed by the addition of Griess reagent to colourimetrically quantify the NO_2^- concentration [25]. The thiobarbituric acid (TBA)-reactive substance levels were measured by the reported methods [26]. Glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), urea nitrogen and creatinine levels were determined using commercial reagents (Transaminase CII-Test Wako were obtained from Wako Pure Chemical Industries Ltd., Osaka, Japan; BUN Kainos and CRE-EN Kainos were obtained from Kainos Laboratories Inc., Tokyo, Japan).

Determination of hepatic and renal TBA-reactive substance levels

The TBA-reactive substance levels in tissue were determined by the method of Uchiyama and Mihara [27] and the protein level was evaluated by the microbiuret method [28] with bovine serum albumin as the standard.

Western blotting

Hepatic and renal tissues were homogenized with ice-cold lysis buffer (pH 7.5) containing 137 mM NaCl,

20 mM Tris-HCl, 1% Tween-20, 10% glycerol, 1 mM PMSF and protease inhibitor mixture DMSO solution. For the determination of NF-κBp65, iNOS, COX-2 and HO-1 protein expressions, 30 μg protein of each sample was electrophoresed via 8 and 12% sodium dodecyl sulfate polyamide gel electrophoresis. Separated proteins were electrophoretically transferred to nitrocellulose membranes, blocked with 5% skim milk solution for 3 h at 4°C and then incubated with primary antibodies overnight at 4°C. After the blots were washed, they were incubated with goat anti-rabbit and/or goat anti-mouse IgG HRP-conjugated secondary antibody for 90 min at room temperature. Each antigen-antibody complex was visualized using ECL Western Blotting Detection Reagents (Amersham, USA) and detected by chemiluminescence with LAS-1000 plus (FUJIFILM, Japan). Band densities were determined by Scion image software (Scion Corporation, USA) and corrected with β-actin.

Statistical analysis

The results for each group are expressed as mean ± SE values. The effect on each parameter was examined using one-way analysis of variance. Individual differences between groups were evaluated using Dunnett's test and those at $p < 0.05$ were considered significant.

Results

Changes in body and tissue weights

There were no significant alterations in body weight changes among the normal, control and 20(*S*)-Rg_3-administered rats before LPS treatment and the body weight changes by LPS treatment were not measured because of severe diarrhoea in rats due to LPS toxicity (data not shown). Liver and kidney weights (g/100 g of body weight) were increased by LPS treatments, but there were no significant changes brought about by 20(*S*)-Rg_3 administrations (Table I).

Biochemical features of serum

The serum NO_2^-/NO_3^- level of LPS-treated control rats was 31.1 μM, which was ~52-times higher than that of normal rats, but it was reduced by 20(*S*)-Rg_3 administrations and showed a significant reduction at the administration dose of 10 mg (Figure 2). The

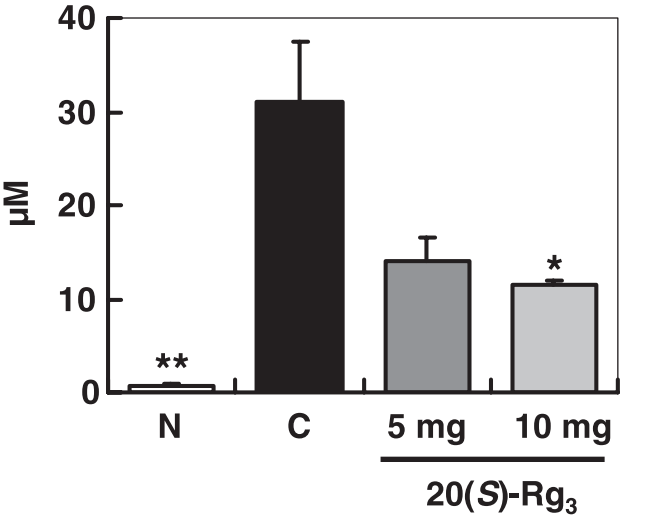

Figure 2. The NO_2^-/NO_3^- levels of serum. N, normal rats; C, LPS-treated control rats. *$p < 0.05$, **$p < 0.01$ compared with LPS-treated control rats.

GOT and GPT levels were significantly increased by LPS treatment, but significantly decreased by 20(*S*)-Rg_3 administrations (Table II). Similarly, creatinine and urea nitrogen levels in LPS-treated control rats were ~2.2- and 2.4-times higher than those of normal rats and significant reductions were shown in creatinine levels, but no significant changes in urea nitrogen levels by 20(*S*)-Rg_3 administrations (Table II). The TBA-reactive substance level was increased from 3.44 nmol/ml to 11.08 nmol/ml by LPS treatment and it was significantly decreased to 4.76 and 4.20 nmol/ml by the 5 and 10 mg 20(*S*)-Rg_3 administrations, respectively (Figure 3A).

Hepatic and renal TBA-reactive substance levels

Hepatic and renal TBA-reactive substance levels in LPS-treated control rats were ~2.5- and 3.0-times increased from normal values, respectively, but these elevated levels were significantly decreased by the 10 mg 20(*S*)-Rg_3 administrations (Figure 3B and C).

Western blot

The NF-κBp65, COX-2, iNOS and HO-1 protein expressions in the liver of LPS-treated control rats were significantly increased by ~1.6, 1.6, 7.2 and 1.8-times, respectively, from normal values. The elevated

Table I. The changes in tissue weights.

Group	Dose (mg/kg body weight/day)	Liver weight (g/100 g of body weight)	Kidney weight (g/100 g of body weight)
Normal	–	3.29 ± 0.08*	0.74 ± 0.02
LPS treatment			
Control	–	3.60 ± 0.06	0.83 ± 0.03
20(*S*)-Rg_3	5	3.52 ± 0.03	0.77 ± 0.01
20(*S*)-Rg_3	10	3.41 ± 0.06	0.76 ± 0.02

Data are expressed as the mean ± SE. *$p < 0.05$ compared with LPS-treated control rats.

Table II. The biochemical features of serum.

Item	Normal	LPS treatment		
		Control	20(*S*)-Rg_3 (5 mg)	20(*S*)-Rg_3 (10 mg)
GOT, Karmen	57.0±1.4**	719.2±86.8	381.0±49.5**	288.7±21.4**
GPT, Karmen	16.9±0.7**	537.1±98.2	166.5±27.8**	89.5±5.9**
Serum creatinine, mg/dl	0.30±0.01*	0.66±0.09	0.40±0.03*	0.39±0.03*
Serum urea nitrogen, mg/dl	16.0±0.2**	38.5±1.8	37.4±1.1	36.7±3.8

Data are expressed as the mean±SE. *$p < 0.05$, **$p < 0.01$ compared with LPS-treated control rats.

NF-κBp65, COX-2 and iNOS protein expressions of LPS-treated control rats were significantly decreased by the 5 and/or 10 mg/kg body weight 20(*S*)-Rg_3 administrations. However, HO-1 protein expressions were more significantly increased by 20(*S*)-Rg_3 administrations than those of LPS-treated control rats (Figure 4). On the other hand, Figure 5 shows the protein expressions related to oxidative stress in kidney tissue. The NF-κBp65, COX-2, iNOS and HO-1 protein expressions in LPS-treated control rats were significantly increased by ~1.7, 2.0, 2.7 and 17.9-times, respectively, from normal values. There were no significant decreases in NF-κBp65 and iNOS brought about by 20(*S*)-Rg_3 administrations, but there were significant reductions in COX-2 and HO-1 levels by the 10 mg/kg body weight 20(*S*)-Rg_3 administration.

Discussion

We have been investigating the main antioxidant components of heat-processed *Panax ginseng* by its free radical scavenging activity-guided fractionation and chemical analysis. As a result, 20(*S*)-Rg_3 was screened out as the strongest $^{\bullet}OH$-scavenging component of *Panax ginseng* by the ferrous metal ion chelating activity of its aglycone structure, 20(*S*)-protopanaxadiol (20(*S*)-PPD), from our previous research [11,17] and dietary nutrients containing metal chelators have received much attention because of their preventive antioxidant activity [23]. To identify the tissue-specific antioxidant effects of 20(*S*)-Rg_3 on an animal model related to oxidative stress, the preventive effect of 20(*S*)-Rg_3 against LPS-induced hepatic and renal injury in rats was compared in this study.

LPS has been shown to increase the constitutive release of $^{\bullet}NO$ by the endothelium and the activity of iNOS enzyme [29,30]. In addition, $^{\bullet}NO$ stimulates H_2O_2 and $O_2^{\bullet-}$ production by mitochondria [31]; in turn, these ROS participate in the up-regulation of iNOS expression via NF-κB activation [32]. ROS also cause the peroxidation of membrane phospholipids, which can alter membrane fluidity and lead to the loss of cellular integrity. Thereby, the impaired activities of mitochondrial enzymes lead to decreased energy levels and organ failure such as of the heart, kidney, lung and liver [21,33]. In this study, there were significant increases in the liver weight, serum NO_2^-/NO_3^-, GOT, GPT, creatinine, urea nitrogen and TBA-reactive substance levels of rats by LPS treatment (Figures 2 and 3, Tables I and II). These parameters show the features of acute inflammation in the liver and kidney by LPS treatment [34–36], but the significant and/or slight decreases in these parameters by 20(*S*)-Rg_3 administrations are suggestive of preventive effects against endotoxin toxicity. Similarly, the elevated TBA-reactive substance levels, one of the oxidative stress biomarkers [37–39], in the liver and kidney of LPS-treated rats were significantly reduced by 20(*S*)-Rg_3 administrations (Figure 3). On the other hand, the ameliorations in hepatic function and damage characterized by serum GOP, GPT and hepatic TBA-reactive substance levels by 20(*S*)-Rg_3 administration were more predominant than those of renal function parameters. Therefore, the preventive effect of 20(*S*)-Rg_3 administration against LPS toxicity was thought to be more predominant in the liver than kidney or LPS-induced oxidative damage was more severe in the kidney and we continuously investigated the protein expressions related to oxidative stresses.

In the comparison of protein expressions related to oxidative stresses in the liver and kidney (Figures 4 and 5), there were significant increases in NF-κBp65, COX-2, iNOS and HO-1 protein expressions in LPS-treated control rats compared to normal rats and the increases in these protein expressions were thought to mediate the LPS-induced ROS generations and tissue damage. The elevated NF-κBp65, COX-2 and iNOS protein expressions in the liver and kidney were decreased by 20(*S*)-Rg_3 administrations, but the decreases in these levels in the kidney were not greater than those of the liver. This tendency was the same as in the serum parameters, except for protein expression of HO-1. HO-1 is readily induced by heme, oxidants, LPS, cytokines, irradiation, heavy metals and stressors, many of which also stimulate iNOS [40]. In addition, HO-1 is believed to play an important role in attenuating tissue injury caused by inflammatory stimuli, and the up-regulation of HO-1 has been shown to protect against LPS-induced cardiovascular collapse or liver damage [41,42]. However, the HO-1 response due to the high

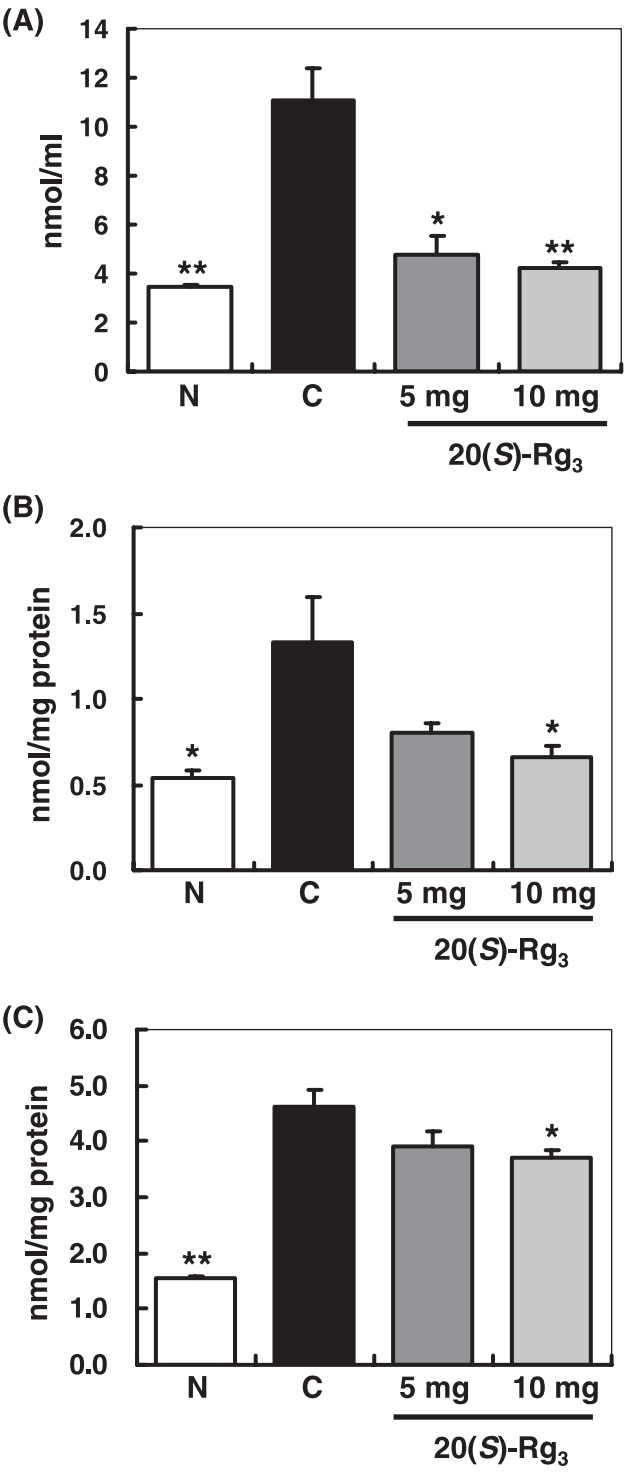

Figure 3. TBA-reactive substance levels in serum (A), liver (B) and kidney (C). N, normal rats; C, LPS-treated control rats. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with LPS-treated control rats.

concentration of $^{\cdot}NO$ donors in the renal tubular epithelial cells is suggestive of cytotoxic effects [40]. The elevated HO-1 levels of the liver and kidney by LPS treatment were more significantly increased and decreased, respectively, by the administration of 20(*S*)-Rg_3. Therefore, the increased HO-1 level in the liver by the 20(*S*)-Rg_3 administration was thought to be mediated by the HO-dependent protective effect against LPS-induced liver damage. However, the decreased HO-1 level in the kidney by 20(*S*)-Rg_3 administrations was thought to suggest decreased oxidative damage in the kidney. This tissue-specific protein expression of HO-1 was thought to be related to the reported tissue-specific gene expression of HO-1 and δ-aminolevulinate synthase (ALAS-N), the rate-limiting enzymes in heme catabolism and heme synthesis, respectively, by LPS treatment. LPS administration significantly induced HO-1 mRNA in the liver and kidney, and ALAS-N mRNA was rapidly decreased in the liver, but significantly increased in the kidney [34]. Therefore, HO-1 induced in the liver by 20(*S*)-Rg_3 administration would reduce the concentration of free heme, a potent prooxidant, as reported by Balla et al. [43], but the significantly increased heme-mediated oxidative injury induces HO-1 in the kidney [44] and its inhibitory effect was thought to result from the antioxidant effect of 20(*S*)-Rg_3.

The most plausible antioxidant mechanism of 20(*S*)-Rg_3 was thought to be through its inhibitory effects on protein expressions related to inflammation such as NF-κBp65, COX-2 and iNOS. Keum et al. [18] also reported that 20(*S*)-Rg_3 inhibits 12-*O*-tetradecanoylphorbol-13-acetate-stimulated activation of NF-κB and extracellular signal-regulated kinase, one of the mitogen-activated protein kinases, as well as COX-2 protein expression in mouse skin and cultured human breast epithelial cells. On the other hand, the stronger effect of 20(*S*)-Rg_3 administration on the liver than kidney was thought to result from the metabolism of ginsenoside. Ginsenoside is known as a prodrug that is activated in the body by intestinal bacterial deglycosylation and fatty acid esterification in the liver, and this process is crucial for its pharmaceutical expression [45]. After deglycosylation, the metabolites of ginsenosides are known to be esterified with fatty acids in the liver without structural variation, and the esterified metabolites are accumulated in the liver. From the study of 20(*S*)-PPD, a metabolite of 20(*S*)-Rg_3, on LPS-induced RAW 264.7 cells, the anti-inflammatory effect of 20(*S*)-PPD was suggested to be mediated by the inactivation of NF-κB, suppression of iNOS and induction of HO-1 [46]. Therefore, accumulated metabolites such as 20(*S*)-PPD in the liver were also thought to show a preventive effect against LPS-induced acute and oxidative tissue damage in this study. In addition, this elevated anti-inflammatory effect of the liver by 20(*S*)-Rg_3 administration was partly thought to result in the reduced oxidative stress in the kidney because the liver acts as both a source and a target of pro- and anti-inflammatory mediators

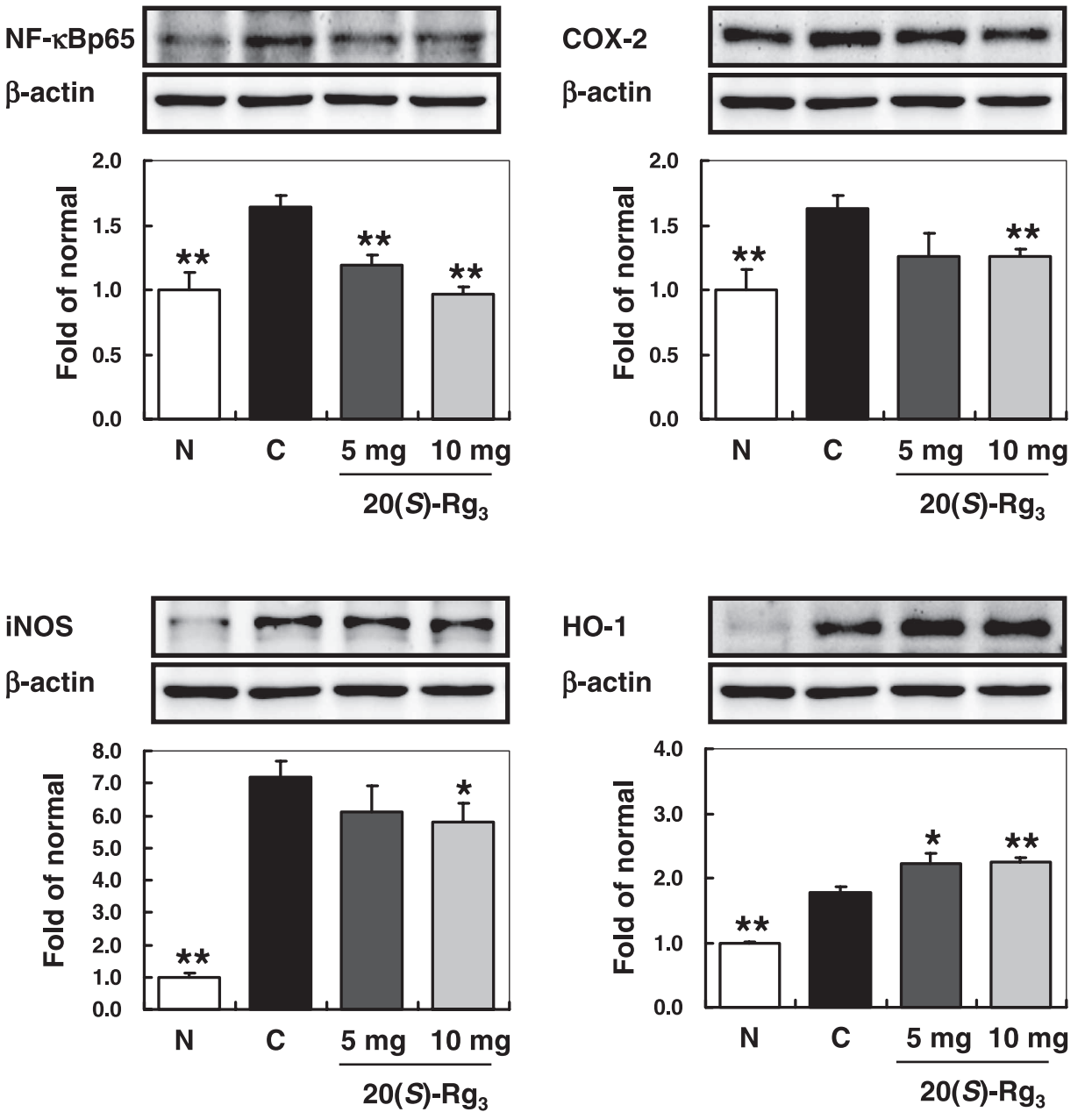

Figure 4. Western blot analysis of liver tissue. N, normal rats; C, LPS-treated control rats. *$p < 0.05$, **$p < 0.01$ compared with LPS-treated control rats.

when LPS is administered [47]. However, we need to conduct further research to investigate these hypotheses using 20(*S*)-PPD or tissue-specific oxidative damage models employing oxidative stress inducers different from LPS in a future study.

In summary, 20(*S*)-Rg_3 administration decreased the elevated serum NO_2^-/NO_3^-, GOT, GPT, creatinine, urea nitrogen and TBA-reactive substance levels of rats by LPS treatment. The TBA-reactive substance, NF-κB, COX-2, iNOS and HO-1 protein expressions in the liver and kidney were significantly increased by LPS treatment. There were significant decreases in TBA-reactive substance, NF-κB, COX-2 and iNOS and an increase in HO-1 protein expressions in the liver by 20(*S*)-Rg_3 administrations. Similarly, there were significant decreases in TBA-reactive substance and COX-2 protein expressions in the kidney by 20(*S*)-Rg_3 administrations, but no significant changes were noted in NF-κB and iNOS protein expressions, and the HO-1 level was significantly decreased by 20(*S*)-Rg_3 administrations. These results suggest the preventive effect of 20(*S*)-Rg_3 against LPS-induced acute oxidative damage in the liver and kidney, and the preventive effect of 20(*S*)-Rg_3 administration against LPS toxicity was thought to be more predominant in the liver than kidney. Therefore, it is clear that 20(*S*)-Rg_3 as an antioxidant can prevent LPS-induced acute oxidative damage.

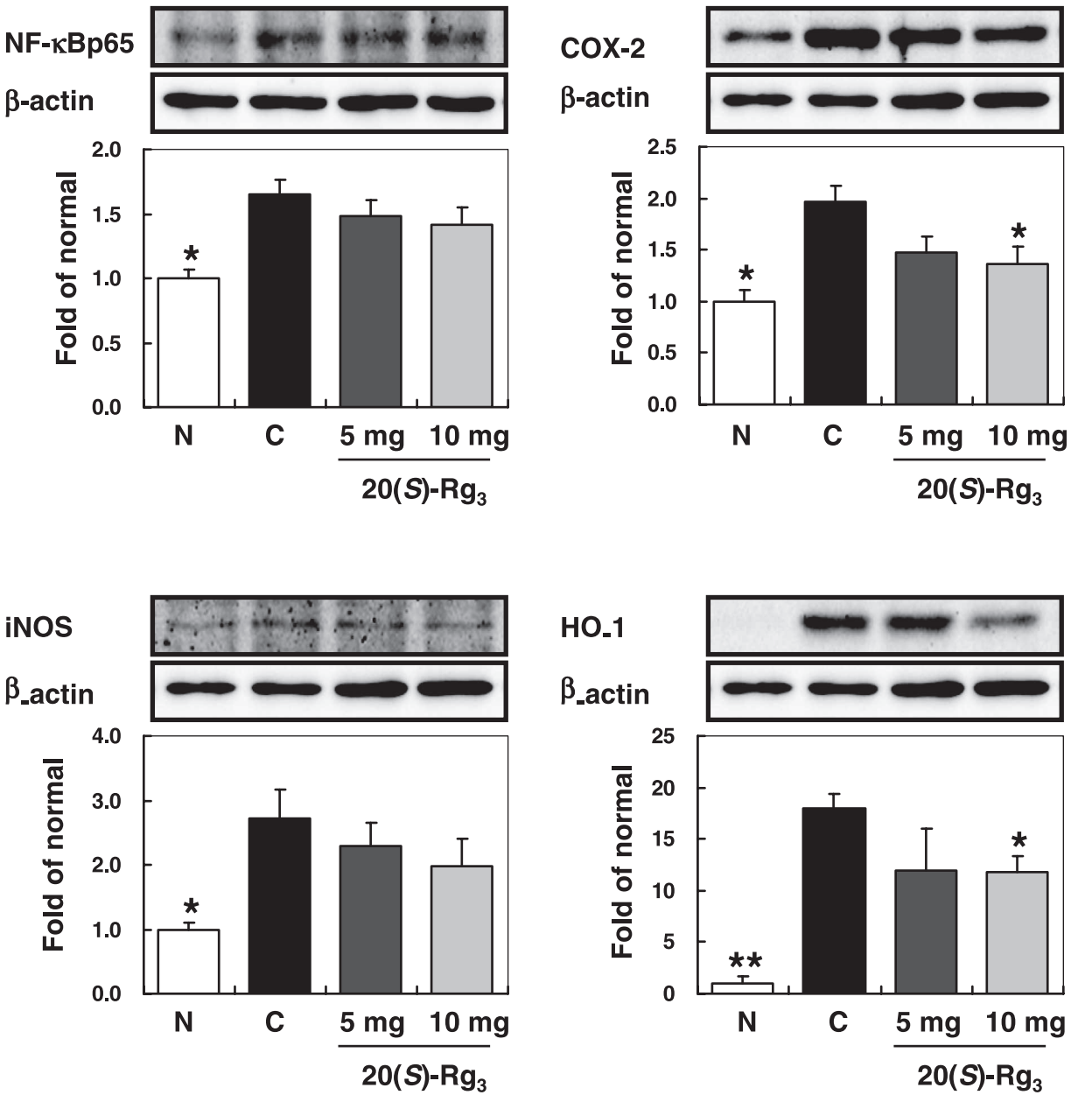

Figure 5. Western blot analysis of kidney tissue. N, normal rats; C, LPS-treated control rats. $*p < 0.05$, $**p < 0.01$ compared with LPS-treated control rats.

References

[1] Xiao PG, Xing ST, Wang LW. Immunological aspects of Chinese medicinal plants as antiageing drugs. J Ethnopharmacol 1993;38:167–175.

[2] Chen X. Cardiovascular protection by ginsenosides and their nitric oxide releasing action. Clin Exp Pharmacol Physiol 1996;23:728–732.

[3] Lee TF, Shiao YJ, Chen CF, Wang LC. Effect of ginseng saponins on beta-amyloid-suppressed acetylcholine release from rat hippocampal slices. Planta Med 2001;67:634–637.

[4] Ivanova T, Han Y, Son HJ, Yun YS, Song JY. Antimutagenic effect of polysaccharide ginsan extracted from *Panax ginseng*. Food Chem Toxicol 2006;44:517–521

[5] Evans P, Halliwell B. Free radicals and hearing. Cause, consequence, and criteria. Ann NY Acad Sci 1999;884:19–40.

[6] Zhang D, Yasuda T, Yu Y, Zheng P, Kawabata T, Ma Y, Okada S. Ginseng extract scavenges hydroxyl radical and protects unsaturated fatty acids from decomposition caused by iron-mediated lipid peroxidation. Free Radic Biol Med 1996;20:145–150.

[7] Kim CS, Park JB, Kim KJ, Chang SJ, Ryoo SW, Jeon BH. Effect of Korea red ginseng on cerebral blood flow and superoxide production. Acta Pharmacol Sin 2002;23:1152–1156.

[8] Kim YK, Guo Q, Packer L. Free radical scavenging activity of red ginseng aqueous extracts. Toxicology 2002;172:149–156.

[9] Kang KS, Yokozawa T, Kim HY, Park JH. Study on the nitric oxide scavenging effects of ginseng and its compounds. J Agric Food Chem 2006;54:2558–2562.

[10] Kang KS, Kim HY, Pyo JS, Yokozawa T. Increase in the free radical scavenging activity of ginseng by heat-processing. Biol Pharm Bull 2006;29:750–754.

[11] Kang KS, Kim HY, Yamabe N, Yokozawa T. Stereospecificity in hydroxyl radical scavenging activities of four ginsenosides

produced by heat processing. Bioorg Med Chem Lett 2006;16:5028–5031.
[12] Kang KS, Kim HY, Yamabe N, Ryoji N, Yokozawa T. Protective effect of sun ginseng against diabetic renal damage. Biol Pharm Bull 2006;29:1678–1684.
[13] Kang KS, Yamabe N, Kim HY, Yokozawa T. Effect of sun ginseng methanol extract on lipopolysaccharide-induced liver injury in rats. Phytomedicine 2007;in press.
[14] Tang SY, Whiteman M, Peng ZF, Jenner A, Yong EL, Halliwell B. Characterization of antioxidant and antiglycation properties and isolation of active ingredients from traditional Chinese medicines. Free Radic Biol Med 2004;36:1575–1587.
[15] Yun TK. Experimental and epidemiological evidence on non-organ specific cancer preventive effect of Korean ginseng and identification of active compounds. Mutat Res 2003;523–524:63–74.
[16] Seo JY, Lee JH, Kim NW, Kim YJ, Chang SH, Ko NY, Her E, Yoo YH, Kim JW, Lee BY, Lee HY, Kim YM, Choi WS. Inhibitory effects of a fermented ginseng extract, BST204, on the expression of inducible nitric oxide synthase and nitric oxide production in lipopolysaccharide-activated murine macrophages. J Pharm Pharmacol 2005;57:911–918.
[17] Kang KS, Yokozawa T, Yamabe N, Kim HY, Park JH. ESR study on the structure and hydroxyl radical scavenging activity relationships of ginsenosides isolated from *Panax ginseng* C. A. Meyer. Biol Pharm Bull 2007;30:917–921.
[18] Keum YS, Han SS, Chun KS, Park KK, Park JH, Lee SK, Surh YJ. Inhibitory effects of the ginsenoside Rg_3 on phorbol ester-induced cyclooxygenase-2 expression, NF-κB activation and tumor promotion. Mutat Res 2003;523–524:75–85.
[19] Tian J, Fu F, Geng M, Jiang Y, Yang J, Jiang W, Wang C, Liu K. Neuroprotective effect of 20(*S*)-ginsenoside Rg_3 on cerebral ischemia in rats. Neurosci Lett 2005;374:92–97.
[20] Lee HU, Bae EA, Han MJ, Kim DH. Hepatoprotective effect of 20(*S*)-ginsenosides Rg_3 and its metabolite 20(*S*)-ginsenoside Rh_2 on *tert*-butyl hydroperoxide-induced liver injury. Biol Pharm Bull 2005;28:1992–1994.
[21] Cadenas S, Cadenas AM. Fighting the stranger-antioxidant protection against endotoxin toxicity. Toxicology 2002;180:45–63.
[22] Vulcano M, Meiss RP, Isturiz MA. Deferoxamine reduces tissue injury and lethality in LPS-treated mice. Int J Immunopharmacol 2000;22:635–644.
[23] Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem 2005;53:1841–1856.
[24] Park JH, Kim JM, Han SB, Kim NY, Surh YJ, Lee SK, Kim ND, Park MK. A new processed ginseng with fortified activity. Advances in ginseng research. Seoul: Korean Society of Ginseng; 1998. p 146–159.
[25] Mabley JG, Suarez-Pinzon WL, Hasko G, Salzman AL, Rabinovitch A, Kun E, Szabo C. Inhibition of poly (ADP-ribose) synthetase by gene disruption or inhibition with 5-iodo-6-amino-1,2-benzopyrone protects mice from multiple-low-dose-streptozotocin-induced diabetes. Br J Pharmacol 2001;133:909–919.
[26] Naito C, Yamanaka T. Lipid peroxides in atherosclerotic diseases. Jpn J Geriat 1978;15:187–191.
[27] Uchiyama M, Mihara M. Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978;86:271–278.
[28] Itzhaki RF, Gill DM. A micro-biuret method for estimating proteins. Anal Biochem 1964;9:401–410.
[29] Salvemini D, Korbut R, Anggard E, Vane J. Immediate release of a nitric oxide-like factor from bovine aortic endothelial cells by *Escherichia coli* lipopolysaccharide. Proc Natl Acad Sci USA 1990;87:2593–2597.
[30] Liu S, Adcock IM, Old RW, Barnes PJ, Evans TW. Lipopolysaccharide treatment *in vivo* induces widespread tissue expression of inducible nitric oxide synthase mRNA. Biochem Biophys Res Commun 1993;196:1208–1213.
[31] Poderoso JJ, Carreras MC, Lisdero C, Riobo N, Schopfer F, Boveris A. Nitric oxide inhibits electron transfer and increases superoxide radical production in rat heart mitochondria and submitochondrial particles. Arch Biochem Biophy 1996;328:85–92.
[32] Han YJ, Kwon YG, Chung HT, Lee SK, Simmons RL, Billiar TR, Kim YM. Antioxidant enzymes suppress nitric oxide production through the inhibition of NF-kappa B activation: role of H_2O_2 and nitric oxide in inducible nitric oxide synthase expression in macrophages. Nitric Oxide 2001;5:504–513.
[33] Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol 1996;271:1424–1437.
[34] Suzuki T, Takahashi T, Yamasaki A, Fujiwara T, Hirakawa M, Akagi R. Tissue-specific gene expression of heme oxygenase-1 (HO-1) and non-specific δ-aminolevulinate synthase (ALAS-N) in a rat model of septic multiple organ dysfunction syndrome. Biochem Pharmacol 2000;60:275–283.
[35] Daull P, Blouin A, Cayer J, Beaudoin M, Belleville K, Sirois P, Nantel F, Chang TM, Battistini B. Profiling biochemical and hemodynamic markers using chronically instrumented, conscious and unrestrained rats undergoing severe, acute controlled hemorrhagic hypovolemic shock as an integrated in-vivo model system to assess new blood substitutes. Vascul Pharmacol 2005;43:289–301.
[36] Hsu BG, Lee RP, Yang FL, Harn HJ, Chen HI. Post-treatment with *N*-acetylcysteine ameliorates endotoxin shock-induced organ damage in conscious rats. Life Sci 2006;79:2010–2016.
[37] Argüelles S, García S, Maldonado M, Machado A, Ayala A. Do the serum oxidative stress biomarkers provide a reasonable index of the general oxidative stress status? Biochim Biophys Acta 2004;1674:251–259.
[38] Voss P, Siems W. Clinical oxidation parameters of aging. Free Radic Res 2006;40:1339–1349.
[39] Wu T, Khoo NH, Zhou F, Day BJ, Parks DA. Decreased hepatic ischemia-reperfusion injury by manganese-porphyrin complexes. Free Radic Res 2007;41:127–134.
[40] Liang M, Croatt AJ, Nath KA. Mechanisms underlying induction of heme oxygenase-1 by nitric oxide in renal tubular epithelial cells. Am J Physiol Renal Physiol 2000;279:728–735.
[41] Takahashi T, Morita K, Akagi R, Sassa S. Heme oxygenase-1: a novel therapeutic target in oxidative tissue injuries. Curr Med Chem 2004;11:1545–1561.
[42] Suliburk JW, Gonzalez EA, Kennison SD, Helmer KS, Mercer DW. Differential effects of anesthetics on endotoxin-induced liver injury. J Trauma 2005;58:711–717.
[43] Balla J, Jacob HS, Balla G, Nath K, Eaton JW, Vercellotti GM. Endothelial-cell heme uptake from heme proteins: Induction of sensitization and desensitization to oxidant damage. Proc Natl Acad Sci USA 1993;90:9285–9289.
[44] Shimizu H, Takahashi T, Suzuki T, Yamasaki A, Fujiwara T, Odaka Y, Hirakawa M, Fujita H, Akagi R. Protective effect of heme oxygenase induction in ischemic acute renal failure. Crit Care Med 2000;28:809–817.
[45] Hasegawa H. Proof of the mysterious efficacy of ginseng: basic and clinical trials: metabolic activation of ginsenoside: deglycosylation by intestinal bacteria and esterification with fatty acid. J Pharmacol Sci 2004;95:153–157.
[46] Lee SH, Seo GS, Ko G, Kim JB, Sohn DH. Anti-inflammatory activity of 20(S)-protopanaxadiol: enhanced heme oxygenase 1 expression in RAW 264.7 cells. Planta Med 2005;71:1165–1170.
[47] Chen T, Zamora R, Zuckerbraun B, Billiar TR. Role of nitric oxide in liver injury. Curr Mol Med 2003;3:519–526.