PHYTOTHERAPY RESEARCH
Phytother. Res. (2009)
Published online in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/ptr.3076

Fermented Ginseng protects Streptozotocin-Induced Damage in Rat Pancreas by inhibiting Nuclear Factor-κB

Hai Dan Yuan[1,2] and Sung Hyun Chung[1]*

[1]Department of Life and Nanopharmaceutical Science, College of Pharmacy, Kyung Hee University, Seoul 130-701, Republic of Korea
[2]Department of Pharmaceutical analysis, College of Pharmacy, Yan Bian University, Yanji, Jilin Province 133000, China

In this study, we investigated the protective effects of fermented ginseng (FG) on hyperglycemia induced by streptozotocin (STZ) in Sprague Dawley rats. FG was administered orally at dose of 250 (FGL) or 500 mg/kg (FGH) for 20 days starting one week before STZ injection. FG restored the plasma insulin levels by 266% and 334% in FGL and FGH, respectively, and resulting in reduction of plasma glucose concentration. Histological observation indicated that STZ-induced destruction of pancreatic islets was protected by FG. Consistent with this observation, FG reduced protein and mRNA levels of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), as determined by Western blotting and RT-PCR, respectively. The molecular mechanism of FG's inhibition of iNOS and COX-2 gene expressions appeared to involve the inhibition of nuclear factor-κB (NF-κB) activation via prevention of inhibitor κB (IκB) phosphorylation and degradation. The cytoprotective effects of FG were also mediated through suppression of extracelluar signal-regulated kinase (ERK) and c-JUN N-terminal kinase (JNK) pathways. Collectively, these results suggest that FG might be used to preserve functional β-cell mass. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: fermented ginseng; streptozotocin; pancreatic islet; NF-κB.

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is an autoimmune disease that is characterized by specific destruction of β-cells in the islets of Langerhans (Atkinson and Maclaren, 1994). At an early stage of the disease, histological finding shows features of insulitis characterized by the infiltration of immune cells, such as T lymphocytes, macrophages, and natural killer cells into pancreas islets (Kwon *et al*., 2006). It is generally thought that immune cells, cytokines, free radicals and production of nitric oxide (NO) have an important role in the pathogenesis of disease (Amirshahrokhi *et al*., 2008). Streptozotocin (STZ) is one of the most widely used diabetogenic agent to induce insulitis in animal models (Szkudelski, 2001). In the STZ-induced hyperglycemic model, STZ can damage pancreatic islet β-cells by activating immune mechanisms and alkylating DNA (Kwon *et al*., 1994). Cumulative evidence suggests that oxygen free radicals or NO mediate deleterious effects of STZ on β-cell dysfunction and destruction (Kwon *et al*., 2006).

Panax ginseng C.A Mayer has been widely used in traditional oriental herbal medicine in many countries and is considered a precious herb medicine (Kim *et al*., 2003). The pharmacological properties of ginseng are mainly attributed to ginsenosides, the active components found in the extracts of different species of ginseng (Nah *et al*., 2007). Up to now, ginsenosides Rb1, Rg1, Rg3, Rh2, Re and compound K have been documented in many studies and found the antidiabetic effects of ginsenosides (Han *et al*., 2007; Lee *et al*., 2007; Chang *et al*., 2008; Park *et al*., 2008; Zhang *et al*., 2008). To find safer and more effective antidiabetic agents from ginseng radix, fermented ginseng (FG) was developed. Recently, FG has been reported to possess hypoglycemic activity (Trinh *et al*., 2007; Kim *et al.,* 2009a). However, virtually no reports have described FG protection against pancreas damage.

In the present study, FG was found to have a preventive effect on STZ-induced pancreatic islets damage in Sprague Dawley rats. To elucidate the mechanism, we examined the effects of FG on the mitogen-activated protein kinase (MAPK) cascade in pancreatic islets: MAPK activation, IκBα phosphorylation and degradation, nuclear translocation of NF-κB, expressions of iNOS and COX-2. MAPKs play critical roles for the activation of NF-κB and subsequently, regulate COX-2 as well as iNOS-NO expression. We demonstrate here that FG inhibits this cascade at the level of MAPK activation, suggesting a potential application for FG in the prevention of pancreatic islets damage.

* Correspondence to: Professor Sung Hyun Chung, Department of Life and Nanopharmaceutical Science, College of Pharmacy, Kyung Hee University, 1 Hoegi-dong, Dongdaemun-ku, Seoul 130-701, Republic of Korea.
E-mail: suchung@khu.ac.kr
Contract/grant sponsor: Kyung Hee University post-doctoral fellowship; Contract/grant number: KHU-20090504; Plant Diversity Research Center of the 21st Century Frontier Program; Contract/grant number: M106KD0110018-09K0401-01810.

MATERIALS AND METHODS

Plant material. FG was kindly supplied by the Central Research Center, Ilhwa Pharmaceutical Co. (Guri-Si,

Received 31 July 2009
Revised 21 October 2009
Accepted 22 October 2009

Copyright © 2009 John Wiley & Sons, Ltd.

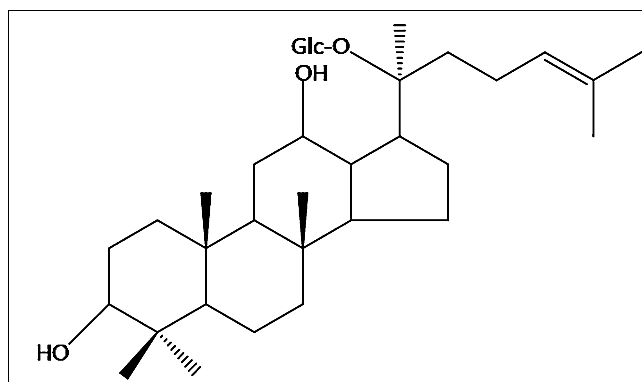

Figure 1. Chemical structure of compound K.

Korea). Briefly, the dried ginseng (1 kg) was extracted from 5 L of ethanol and concentrated with a speedvac. The dry ginseng extract was incubated with an enzyme solution containing 1.5% β-galactosidase (Sigma-Aldrich, St Louis, MO, USA) at 50 °C for 72 h and adjusted the chilled solution with citric acid to pH 5.6, and finally incubated the solution with cellulose (Sigma-Aldrich, St Louis, MO, USA) for 48 h at 37 °C until diol ginsenosides such as Rb_1, Rc, Rb_2, and Rd were changed to compound K. The chemical structure of compound K is depicted in Fig. 1.

Animal. Six-week-old male Sprague Dawley rats (310–350 g) were purchased from ORIENT BIO (Seoul, Korea). All animals were acclimatized to the laboratory environment for 2 weeks before the experiment. Rats were allowed free access to drinking water and food under constant room temperature (22 ± 2 °C) and humidity (50 ± 10%) conditions with an automatic 12-h light/dark cycle. Experimental protocols were approved by the Institutional Animal Ethics Committee of the Kyung Hee University. Rats were randomly divided into 4 groups: normal control group (NC); streptozotocin–induced diabetic group (STZ); fermented ginseng 250 mg/kg group (FGL); and fermented ginseng 500 mg/kg group (FGH). A single dose of 65 mg/kg STZ prepared in citrate buffer (0.1 M, pH 4.0) was injected intraperitoneally to induce diabetes. Diabetes was confirmed after 48 h of STZ injection, and the rats showing plasma glucose levels of more than 250 mg/dl were selected and used for the present study. In the treatment groups, rats were orally administered once a day for 20 days starting one week before STZ injection.

Determination of serum parameters. After 20 days of treatment, blood samples were collected through tail vein; plasma glucose and insulin levels were measured. Plasma glucose and insulin concentrations were determined using enzymatic GOD-PAP (glucose oxidase peroxidase) diagnostic kit (Asan Pharmaceutical Co., Seoul, Korea) and a mouse insulin enzyme immunoassay kit (Shibayagi Co., Gunma, Japan), respectively.

Histological analysis. The pancreas tissue was removed and fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned with 5 μm thickness (Leica, Wetzlar, Germany), and then stained with hematoxylin-eosin for microscopic assessment (Olympus, Tokyo, Japan).

Western blot analysis. After sacrifice, the pancreases were immediately removed and instantly soaked in liquid nitrogen and stored at −70 °C. Protein extracts were prepared using a protein extraction kit (Intron Biotechnology Inc., Seoul, Korea). Lysates (40 μg) were electroblotted onto a nitrocellulose membrane following separation on a 8% SDS-polyacrylamide gel electrophoresis. Blotted membranes were incubated for 1 h with blocking solution (tris-bufferd saline/Tween 20, TBST) containing 5% skim milk (w/v) at room temperature, followed by incubation overnight at 4 °C with 1:2,000 diluted iNOS, COX-2, ERK, p-ERK, JNK, p-JNK, IκBα, p-IκBα, β-actin primary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Membranes were washed 4 times with 0.1% TBST and incubated with 1:3,000 diluted horseradish peroxidase-conjugated goat anti-rabbit or donkey anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA) secondary antibody for 1 h at room temperature. Membranes were washed 4 times in TBST and then developed by ECL (Amersham, Uppsala, Sweden).

RNA preparation and Reserve Transcription-Polymerase Chain Reaction (RT-PCR). Total mRNA was isolated from rat pancreas using an Easy-Blue kit (Intron Biotechnology Inc., Seoul, Korea) according to the manufacturer's instruction. From each sample, total RNA (10 μg) was reverse transcribed into cDNA using Moloney murine leukemia virus transcriptase and Oligo (dT) 15 primers (Promega, Madison, WI, USA) as primers. The cDNA fragment was amplified by PCR using the following specific primers: iNOS mRNA (F): 5′-ATGGCTTGCCCCTGGAAFT-3′; (R): 5′-GTACTTGGGATGCTCCATGGTCA-3′; COX-2 mRNA (F): 5′-ATGCTCTTCCGAGCTGTGCT-3′; (R): 5′-TTACAGCTCAGTTGAACGCCTTTT-3′; CPN mRNA (F): 5′-ATGGTCAACCCACCGTG-3′; (R): 5′-TTAGAGTTGTCCACAGTTCGGAGA-3′. PCR was initiated a thermal cycle programmed at 95 °C for 5 min, 95 °C for 30 sec, 57 °C for 30 sec, 72 °C for 30 sec, and amplified for 30 cycles. The RT-PCR products were electrophoresed on 1% agarose gels and visualized by 0.5 ug/ml ethidium bromide staining and scanning densitometry was performed with I-MAX Gel Image analysis system (Core-Bio, Seoul, Korea). CPN was amplified as a control gene.

Nuclear extraction and Electrophoretic Mobility Shift Assay (EMSA). Nuclear extract was prepared using a nuclear extraction kit (Fermentas Inc., Glen Burnie, MD, USA). IκBα and p-IκBα in the cytoplasmic fraction were analyzed via western blotting. Nuclear extract (10 μg) was mixed with double-stranded NF-κB oligonucleotide 5′-AGTTGAGGGGACTTTCCCAG-GC3′ end-labeled by [γ-^{32}P] dATP (underlying indicates α κB consensus sequence or a binding site for NF-κB/cRel homodimeric and heterodimeric complex). The binding reactions were performed at 37°C for 30 min in 30 μl of reaction buffer containing 10 mM Tris–HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA, 4% glycerol, 1 μg of poly (dI-dC) and 1 mM DTT. The specificity of binding was examined by competition with the 80-fold unlabeled oligonucleotide. DNA/nuclear protein complexes were separated from the unbound DNA probe on native 5% polyacrylamide gels at 100 V in 0.5 × TBE (5 mM Tris, 1 mM borate, 5 mM EDTA) buffer. The gels were

Copyright © 2009 John Wiley & Sons, Ltd.

DOI: 10.1002/ptr

vacuum dried for 1 h at 80°C and exposed to X-ray film at −70°C for 24 h.

Statistical analysis. Results were expressed as mean ± SEM and differences between groups were analyzed using Student's *t*-test. Statistical significance was considered at $p < 0.05$.

RESULTS

Effect of FG on plasma glucose and insulin levels

Twenty days after STZ injection, plasma glucose levels were elevated (340.2 ± 21.1 mg/dl) as compared to the control rats (175.1 ± 7.8 mg/dl). There was a marked decline in the insulin levels of STZ-treated rats compared to age matched control rats (Fig. 2B). Chronic treatment of FG dose dependently preserved the plasma insulin levels. Consequently, plasma glucose levels in FG-treated groups were reduced by 24.5% and 20.7% in FGL and FGH, respectively, compared to the STZ control group (Fig. 2A).

FG protects pancreatic islets

To evaluate the protective effect of FG on STZ-induced pancreatic islets damage, histological examination was performed. The STZ group rats exhibited definitive β-cell loss and degeneration of islet, whereas rats treated with FG preserved islet architecture (Fig. 3). This observation demonstrated that STZ-induced destruction of β-cell and the degree of inflammation were ameliorated by FG in pancreas.

Effects of FG on expressions of iNOS, COX-2 protein and mRNA

Effects of FG on the expressions of iNOS and COX-2 protein and mRNAs were determined by western blot analysis and RT-PCR, respectively. As shown in Fig. 3, iNOS and COX-2 protein and mRNA expressions were markedly increased in rat pancreas treated with STZ alone, whereas rats treated with FG showed suppressed expressions of the protein and mRNA levels of iNOS and COX-2 (Figs 4A and 4B). This result indicates that the protective effect of FG against STZ-induced pancreas damage is due to the suppression of iNOS and COX-2 gene expressions.

Effect of FG on NF-κB activation

Because NF-κB is implicated in the transcriptional regulation of iNOS and COX-2 expressions, EMSA was performed to examine whether FG influences NF-κB activation. As shown in Fig. 4A, nuclear extract from

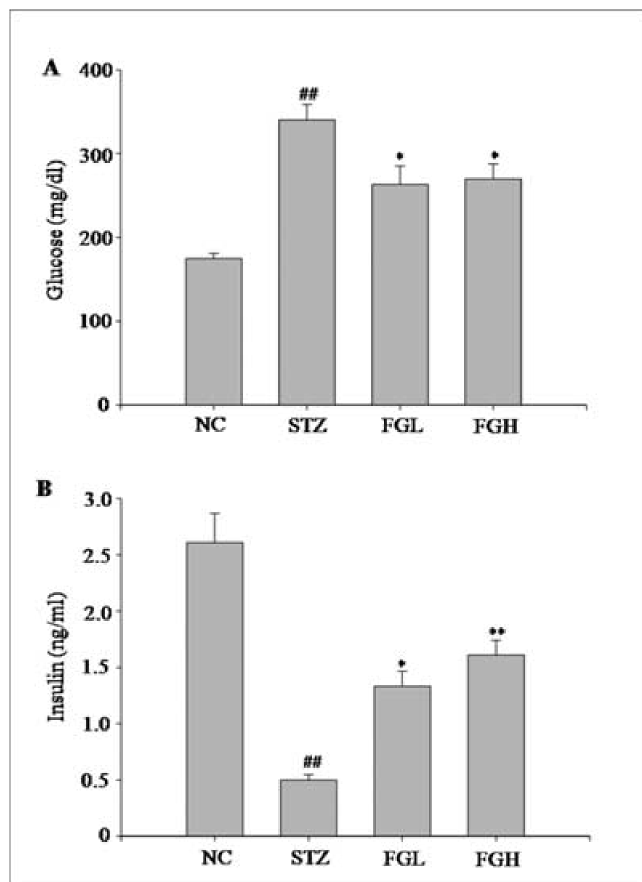

Figure 2. Effects of FG on plasma glucose (A) and insulin (B) levels in STZ-induced diabetic rats. Values represent the mean ± SE (n = 6). *p<0.05, **p<0.01 vs. STZ; ##p<0.01 vs. NC.

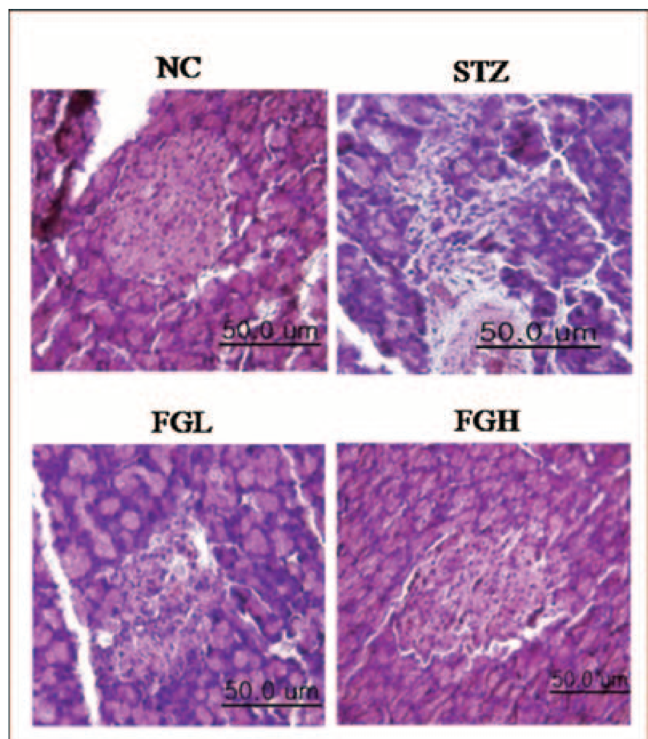

Figure 3. Histological profiles for pancreatic islets in normal rats (NC), diabetic rats (STZ), FG 250 mg/kgtreated rats (FGL) and FG 500 mg/kg-treated rats (FGH). Relatively well documented pancreatic islets and tightly arranged islet cells were observed in normal rats. Severe necrosis and mild atrophy of islets were found in diabetic rats, which were improved after treatment with FG. Stained sections showing islet morphology in sections stained with H&E, ×400.

Copyright © 2009 John Wiley & Sons, Ltd.

DOI: 10.1002/ptr

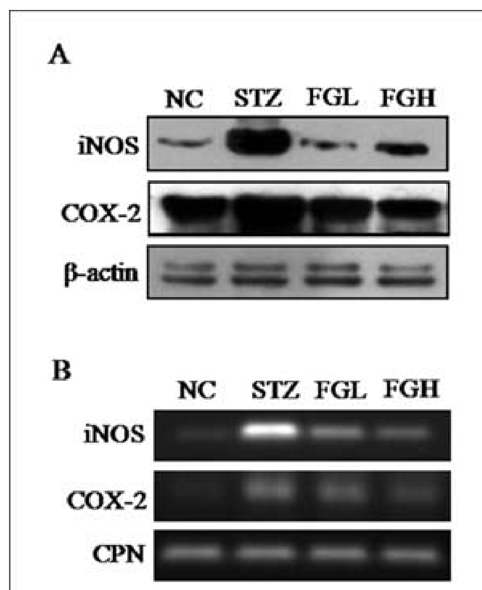

Figure 4. Effects of FG on iNOS and COX-2 protein (A) and mRNA (B) expression in rat pancreas. A single dose of 65 mg/kg STZ prepared in citrate buffer (0.1 M, pH 4.0) was injected intraperitioneally to induce diabetes. Diabetes was confirmed after 48 h of STZ injection, and the rats showing plasma glucose levels more than 250 mg/dl were selected and used for the present study. In the treatment groups, rats were orally administered once a day for 20 days starting one week before STZ injection. After treatment with STZ for two weeks, iNOS and COX-2 protein (A) and mRNA (B) expressions in rat pancreas were measured by Western blot and RT-PCR as described in Materials and Methods.

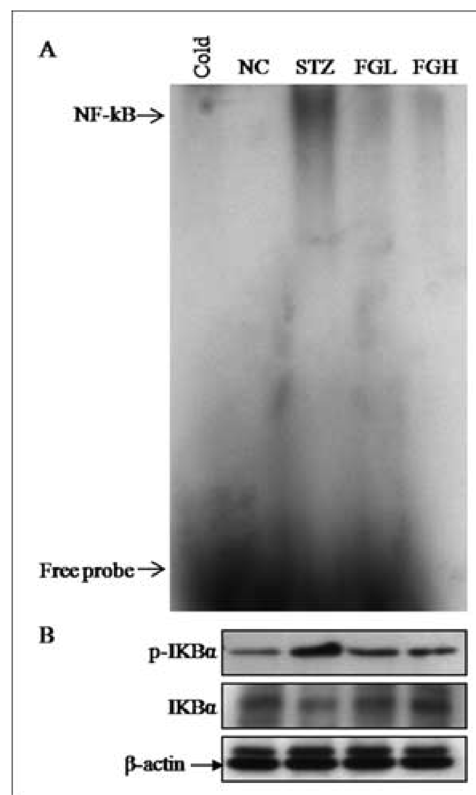

Figure 5. Effects of FG on translocation of NF-κB from cytosol to the nucleus (A) and IκBα degradation (B) in pancreas. At the end of treatment of FG, DNA binding of NF-κB was analyzed by EMSA, and IκBα degradation in cytosol was determined by Western blot as described in Materials and Methods.

STZ-injected rat pancreas showed an increase in NF-κB DNA binding activity, and this NF-κB DNA binding was markedly suppressed in FG-treated rats. Next, to determine whether FG's inhibition of nuclear translocation of NF-κB is due to an effect on IκBα degradation, the cytoplasmic levels of IκBα were determined by western blot analysis. The result showed that STZ-induced IκBα degradation was significantly prevented by FG in a dose-dependent manner (Fig. 4B). Because NF-κB is activated by IκBα degradation after phoshporylation of IκBα at the serine residues (May and Ghosh, 1998), FG also inhibited the STZ-induced phosphorylation of IκBα protein expression (Fig. 5B).

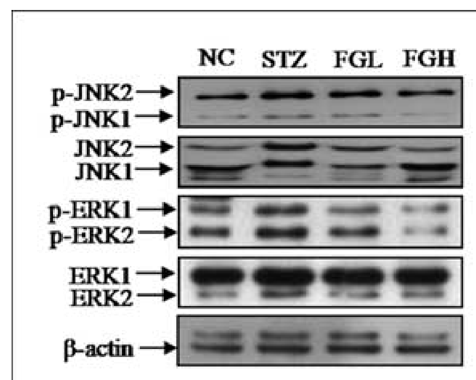

Figure 6. Effects of FG on phosphorylations of MAPKs in rat pancreas. At the end of treatment of FG, p-ERK, ERK, p-JNK, JNK protein expressions in rat pancreas were measured by Western blot as described in Materials and Methods.

Effect of FG on ERK1/2 and JNK phosphorylation

MAPKs are known to be play important roles in the activation of NF-κB (Minden *et al*., 1994; van den Berghe *et al*., 1998). To investigate whether the inhibition of NF-kB activation by FG is mediated by MAPKs pathway, we examined the effects of FG on the STZ-induced phosphorylations of ERK1/2 and JNK in rat pancreas by western blot analysis. As shown in Fig. 6, FG suppressed the STZ-induced activations of ERK and JNK in dose-dependent manners. The results suggest that FG's inhibition of NF-κB activation is due to inhibitions of ERK and JNK phosphorylation.

DISCUSSION

Ginseng is widely used in oriental medicine to treat various diseases. The pharmacological effects of ginseng are mostly attributed to ginsenoside and many studies documented the antidiabetic activity of ginsenoside. (Vuksan and Sievenpiper, 2005; Han *et al*., 2006; 2007; Shang *et al*., 2007; Park *et al*., 2008). Compound K (CK), an intestinal metabolite of panaxadiol ginsenosides, is considered the main active constituent in FG. Recently, our laboratory reported that CK significantly decreased

Copyright © 2009 John Wiley & Sons, Ltd.

DOI: 10.1002/ptr

the fasting blood glucose levels in C57BL/KSJ db/db mice through insulin secretion and improved insulin resistance (Han *et al*., 2007). In addition, CK was shown to activate AMPK and affect lipid metabolism in insulin-resistant human HepG2 cells (Kim *et al*., 2009b). We also reported that FG showed hypoglycemic activity in C57BL/KSJ *db/db* mice through AMPK activation (Kim *et al*., 2009a). Park *et al*. (2005) showed the effects of ginsenoside Rb1 and CK on NO and prostaglandin E2 synthesis and CK was shown to reduce the expression levels of iNOS and COX-2 in RAW 264.7 cells. However, there were no reports documenting the protection activity of FG against STZ-induced islets damage.

In the STZ-induced hyperglycemic rats, diabetes arises from the irreversible destruction of pancreatic β-cells due to free radicals and NO, resulting in degranulation and reduction of insulin secretion (Nathan, 1992). In the present study, pretreatment with FG significantly reduced STZ-induced high blood glucose levels, and markedly elevated plasma insulin levels in a dose-dependent manner (Fig. 1). Subsequently, histological analysis of pancreatic tissue showed that the islets of STZ-injected control group revealed degenerative and necrotic changes, and islets shrinkage. However, FG was prevented from destroying islets caused by STZ (Fig. 2). These results showed that STZ-induced pancreatic β-cell damage was ameliorated by FG, conserved of insulin contents, and consequently lowered plasma glucose levels.

Inflammation is associated with a large number of mediators such as iNOS and COX-2 (O'Neill, 2006), which are regulated by NF-κB transcription factor (Baeuerle and Baltimore, 1996). To explore the action mechanisms of FG, we examined the expression levels of iNOS and COX-2 protein and mRNA. We found that FG inhibits iNOS and COX-2 protein expressions in STZ-induced rat pancreas, and it probably acts at the transcriptional level, as evidenced by reductions in their mRNA levels (Fig. 3). NF- κB is known to play a critical role in the regulation of cell survival genes, and coordinates the expressions of pro-inflammatory enzymes and cytokines (Karin, 1999). In non-stimulated cells, NF-κB is present in the cytosol as a homodimer or heterodimer, and in particular is linked to the inhibitory proteins, such as IκB (Baeuerle and Baltimore, 1996; Zhou *et al*., 2008). The activation of NF-κB results in the phosphorylation, ubiquitination, and proteasome-mediated degradation of IκB proteins, followed by the nuclear translocation and DNA binding of NF-κB (Chun *et al*., 2003). As shown in Fig. 4, FG inhibited the nuclear translocation of the p65 protein and activation of NF-κB. Additionally, western blot showed that FG prevented the degradation and resynthesis of IκBα protein. These results revealed that pretreatment with FG inhibits the STZ-induced expressions of iNOS and COX-2 through inactivation of NF-κB resulting in reduction of IκBα degradation and phosphorylation in rat pancreas.

One of the most extensively investigated intracellular signaling cascades involved in pro-inflammatory responses is the MAPK pathway. MAPK is known to regulate NF-κB activation by multiple mechanisms (Garrington and Johnson, 1998; van den Berghe *et al*., 1998). Accumulating evidence indicates that NF-κB activation is modulated by ERK1/2 and JNK (Chun *et al*., 2003; Zhou *et al*., 2008). To further investigate the mechanisms of FG, effects on phosphorylations of ERK and JNK were examined. As shown in Fig. 5, FG dose-dependently inhibited the phosphorylations of ERK and JNK in pancreas. These results suggested that FG's inhibition of NF-κB activation might be due to inhibition of ERK and JNK phosphorylations.

In summary, our results suggest that FG prevented STZ-induced pancreas β-cell damage through the suppression of iNOS and COX-2 expression via downregulation of the MAPK signal pathway and inactivation of NF-κB, indicating that FG may have a beneficial effect when used to prevent the progress of Type 1 diabetes.

Acknowledgments

This work was supported by a grant from the Kyung Hee University post-doctoral fellowship in 2009 (KHU-20090504) and by a research grant (M106KD0110018-09K0401-01810) from the Plant Diversity Research Center of the 21st Century Frontier Program.

REFERENCES

Amirshahrokhi K, Dehpour AR, Hadjati J, Sotoudeh M, Ghazi-Khansari M. 2008. Methadone ameliorates multiple-low-dose streptozotocin-induced type 1 diabetes in mice. *Toxicol Appl Pharmacol* **232**: 119–124.

Atkinson MA, Maclaren NK. 1994. The pathogenesis of insulin-dependent diabetes mellitus. *N Engl J Med* **331**: 1428–1436.

Baeuerle PA, Baltimore D. 1996. NF-kappa B: ten years after. *Cell* **87**:13–20.

Chang Y, Huang WJ, Tien LT, Wang SJ. 2008. Ginsenosides Rg1 and Rb1 enhance glutamate release through activation of protein kinase A in rat cerebrocortical nerve terminals (synaptosomes). *Eur J Pharmacol* **578**: 28–36.

Chun KS, Keum YS, Han SS, Song YS, Kim SH, Surh YJ. 2003. Curcumin inhibits phorbol ester-induced expression of cyclooxygenase-2 in mouse skin through suppression of extracellular signal-regulated kinase activity and NF-kappaB activation. *Carcinogenesis* **24**: 1515–1524.

Garrington TP, Johnson GL. 1998. Organization and regulation of mitogen-activated protein kinase signaling pathways. *Curr Opin Cell Biol* **11**: 211–218.

Han GC, Ko SK, Sung JH, Chung SH. 2007. Compound K enhances insulin secretion with beneficial metabolic effects in db/db mice. *J Agric Food Chem* **55**: 10641–10648.

Han KL, Jung MH, Sohn JH, Hwang JK. 2006. Ginsenoside 20(S)-protopanaxatriol (PPT) activates peroxisome proliferator-activvated receptor γ (PPARγ) in 3T3-L1 adipocytes. *Biol Pharm Bull*. **29**: 110–113

Karin M. 1999. How NF-kappaB is activated: the role of the IkappaB kinase (IKK) complex. *Oncogene*. **18**: 6867–6874.

Kim DH, Jung JS, Moon YS, Sung JH, Suh HW, Kim YH, Song DK. 2003. Inhibition of intracerebroventricular injection stress-induced plasma corticosterone levels by intracerebroventricularly administered compound K, a ginseng saponin metabolite, in mice. *Biol Pharm Bull* **26**: 1035–1038.

Kim DY, Park JS, Yuan HD, Chung SH. 2009a. Fermented ginseng attenuates hepatic lipid accumulation and hyperglycemia through AMPK activation. *Food Sci Biotechnol* **18**: 172–178.

Kim DY, Yuan HD, Chung IK, Chung SH. 2009b. Compound K, Intestinal metabolite of ginsenoside, attenuates hepatic

Copyright © 2009 John Wiley & Sons, Ltd.

lipid accumulation via AMPK activation in human hepatoma cells. *J Agric Food Chem* **57**: 1532–1537.

Kwon KB, Kim E K, Jeong ES, Lee YH, Lee YR, Park JW, Ryu DG, Park BH. 2006. Cortex cinnamomi extract prevents streptozotocin- and cytokine-induced beta-cell damage by inhibiting NF-kappaB. *World J Gastroenterol* **12**: 4331–4337.

Kwon NS, Lee SH, Choi CS, Kho T, Lee HS. 1994. Nitric oxide generation from streptozotocin. *FASEB J* **8**: 529–533.

Lee WK, Kao ST, Liu IM, Cheng JT. 2007. Ginsenoside Rh2 is one of the active principles of Panax ginseng root to improve insulin sensitivity in fructose-rich chow-fed rats. *Horm Metab Res* **39**: 347–354.

May MJ, Ghosh S. 1998. Signal transduction through NF-kappa B. *Immunol Today* **19**: 80–88.

Minden A, Lin A, McMahon M, Lange-Carter C, Dérijard B, Davis RJ, Johnson GL, Karin M. 1994. Differential activation of ERK and JNK mitogen-activated protein kinases by Raf-1 and MEKK. *Science* **266**: 1719–1723.

Nah SY, Kim DH, Rhim H. 2007. Ginsenosides: Are Any of them Candidates for Drugs Acting on the Central Nervous System? *CNS Drug Rev* **13**: 381–404.

Nathan C. 1992. Nitric oxide as a secretory product of mammalian cells. *FASEB J* **6**: 3051–3064.

O'Neill LA. 2006. Targeting signal transduction as a strategy to treat inflammatory diseases. *Nat Rev Drug Discov* **5**: 549–563.

Park EK, Shin YW, Lee HU, Kim SS, Lee YC, Lee BY, Kim DH. 2005. Inhibitory effect of ginsenoside Rb1 and compound K on NO and prostaglandin E2 biosyntheses of RAW234.7 cells induced by lipopolysaccharide. *Biol Pharm Bull* **28**: 652–656.

Park MW, Ha J, Chung SH. 2008. 20(S)-ginsenoside Rg3 enhances glucose-stimulated insulin secretion and activates AMPK. *Biol Pharm Bull* **31**: 748–751.

Shang W, Yang Y, Jiang B, Jin H, Zhou L, Liu S, Chen M. 2007. Ginsenoside Rb1 promotes adipogenesis in 3T3-L1 cells by enhancing PPARγ2 and C/EBPα gene expression. *Life Sci* **80**: 618–625

Szkudelski T. 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res* **50**: 536–546.

Trinh HT, Han SJ, Kim SW, Lee YC, Kim DH. 2007. Bifidus fermentation increases hypolipidemic and hypoglycemic effects of red ginseng. *J Microbiol Biotechnol* **17**: 1127–1133.

van den Berghe W, Plaisance S, Boone E, De Bosscher K, Schmitz ML, Fiers W, Haegeman G. 1998. p38 and extracellular signal-regulated kinase mitogen-activated protein kinase pathways are required for nuclear factor-kappaB p65 transactivation mediated by tumor necrosis factor. *J Biol Chem* **273**: 3285–3290.

Vuksan V, Sievenpiper JL. 2005. Herbal remedies in the management of diabetes: Lessons learned from the study of ginseng. *Nute Metab Cardiovas* **15**: 149–160.

Zhang Z, Li X, Lv W, Yang Y, Gao H, Yang J, Shen Y, Ning G. 2008. Ginsenoside Re reduces insulin resistance through inhibition of c-Jun NH2-terminal kinase and nuclear factor-kappaB. *Mol Endocrinol* **22**, 186–195.

Zhou HY, Shin EM, Guo LY, Youn UJ, Bae KW, Kang SS, Zou LB, Kim YS. 2008. Anti-inflammatory activity of 4-methoxyhonokiol is a function of the inhibition of iNOS and COX-2 expression in RAW 264.7 macrophages via NF-kappaB, JNK and p38 MAPK inactivation. *Eur J Pharmacol* **586**: 340–349.

Copyright © 2009 John Wiley & Sons, Ltd.

DOI: 10.1002/ptr