Protective Effects of Isoliquiritigenin Against Methamphetamine-Induced Neurotoxicity in Mice

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Abstract. Isoliquiritigenin (ISL) suppresses cocaine-induced extracellular dopamine levels and has a neuroprotective effect in cocaine-treated rat brain. Here, we examine the effect of ISL on methamphetamine-induced striatal neurotoxicity. Repeated injections of methamphetamine cause the loss of striatal dopamine transporter (DAT) and tyrosine hydroxylase (TH). Intraperitoneal injection of ISL prior to methamphetamine injection significantly prevented methamphetamine-induced reduction of DAT and TH. ISL also suppressed methamphetamine-induced activation of glial cells. Moreover, ISL impeded the expression of nitric oxide synthase and the activation of NF-κB through blockage of its phosphorylation. Our results suggest that ISL protects against methamphetamine-induced neurotoxicity by inhibition of NF-κB activation.

Keywords: methamphetamine, neurotoxicity, isoliquiritigenin

Methamphetamine (METH) is one of the substituted amphetamines with central nervous stimulant effects. It may cause long-lasting neural damages, leading to the degeneration of dopaminergic and serotonergic nerve terminals in multiple brain areas. Thus, it leads to reductions of dopaminergic function such as long-term decreases in tyrosine hydroxylase (TH) activity and dopamine transporter (DAT) (1). The mechanism responsible for METH-induced damages has been under intense study, yet much remains to be elucidated. However, leading hypotheses revolve around oxidative stress (2). Excess dopamine (DA) by METH can be oxidized to form highly reactive DA quinones and reactive oxygen species, leading to increase in oxidative stress (3). Oxidative stress could account for many of the effects of METH on the DA nerve endings such as inhibition of TH activity (4) and reduction of DAT (5). A number of studies have hinted to a role for oxidative stress in METH-induced damages. For example, administration of antioxidants such as ascorbic acid has shown attenuation of METH-induced neurotoxicity (6).

Isoliquiritigenin (ISL) is a flavonoid with a chalcone structure and an active component of licorice (Glycyrrhiza radix). Flavonoids are a class of plant secondary metabolite whose function is commonly known for antioxidant activity. Some flavonoids have been reported to protect against METH-induced neurotoxicity. For instance, baicalein, a flavonoid from Scutellaria baicalensis, has been demonstrated to attenuate METH-induced loss of DAT in mouse striatum by inhibition of nitric oxide synthase (NOS) overexpression (7). ISL has a variety of cellular or physiological functions such as being an antioxidant. Recently, effects of ISL on physiological or behavioral changes in response to psychostimulants have been reported. It has an inhibitory effect on cocaine-induced DA release in the nucleus accumbens of rat brain (8). ISL suppresses cocaine-induced behavioral changes such as locomotor activity. In addition, ISL modulated the expression of genes related to cell damages and oxidative stress. It has also been shown to protect the glial cells from cocaine treatment (9). Based on previous studies, we performed the present investigation to evaluate the neuroprotective effect of ISL in the METH-neurotoxicity mouse model.

Male C57/BL6 mice (Hyuchang, Seoul, South Korea) weighing 20 – 25 g were used in the present study. All mice were kept on ad libitum food and water and
maintained on a 12-h light/dark cycle throughout the course of the study. All animal experiments were approved by the Institutional Animal Care and Use Committee and were accomplished in accordance with the provisions of the NIH Guideline for the Care and Use of Laboratory Animals. METH hydrochloride obtained from the Korea Food & Drug administration was dissolved in saline. ISL was provided by Dr. Kim (Daegu Haany University, Daegu, South Korea) and dissolved in vehicle (TWEEN-80 : polyethylene glycol : ethanol : water = 70:10:10:10). Mice were injected repeatedly with METH (3 mg/kg × 4, i.p. with 2-h intervals) or saline. Intraperitoneal injections of ISL (20 mg/kg × 3) or vehicle were performed 30 min prior to the 1st and 3rd METH/saline injections and 24 h after the 1st ISL injection. Dose–response curves for ISL activity on DA release were previously reported, and the dose used for this study was derived from this prior work (8, 9). Rectal temperature was recorded 30 min before the 1st and after the final METH injection, respectively. Mice were sacrificed 3 days after the last injection of METH or saline for immunohistochemistry and Western blotting. Mice were transcardially perfused with saline followed by a fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) under pentobarbital sodium anesthesia (50 mg/kg, i.p.). After the perfusion, the brains were rapidly removed from the skull, pre-fixed for 24 h in a fixative containing 4% paraformaldehyde in 0.1 M PB (pH 7.4), and then cryoprotected in 15% sucrose in PB for 48 h. Brain, frozen with OCT compound (Thermo Inc., Walthan, MA, USA), was cut coronally on a cryostat at levels containing the mid-striatum (+0.6 to -0.7 mm from the bregma) at 30-μm thickness. The sections were soaked in 0.5% H₂O₂ in 10 mM PBS containing 0.2% Triton X-100 (PBST) for 30 min at room temperature. After washing with PBST (5 min × 5), the sections were incubated in 1% normal goat serum for 30 min. The sections were exposed to rat anti-DAT monoclonal antibody (diluted 1:1000 in PBST; Chemicon, Billerica, MA, USA) or mouse anti-glial fibrillary acidic protein (GFAP) monoclonal antibody (diluted 1:1000 in PBST, Chemicon) for 18 h at 4°C. After incubation with the primary antibody, sections were washed for 5 min × 5 in PBST before incubation with biotinylated secondary antibody (diluted 1:1000 in PBST; Vector Laboratories, Burlingame, CA, USA) for 2 h at room temperature. Following washes in PBST (10 min × 3), the sections were incubated with the avidin–biotin peroxidase complex (diluted 1:2000, Vector Laboratories) for 1 h at room temperature. Immunopositive signals were visualized by 3,3'-diaminobenzidine, nickel, and H₂O₂. Brain tissues were homo-
genized in lysis buffer containing 50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 2 mM EDTA, 1 mM EGTA, and 1 mM phenylmethylsulfonyl fluoride (PMSF) including protease inhibitor cocktail. Homogenates were kept 20 min on ice and centrifuged 15 min at 12,000 rpm at 4°C. Total proteins were fractionated by 10% – 12% gel electrophoresis and electrophoretically transferred to a PVDF membrane. Membranes were incubated with one of the following primary antibodies: GFAP antibody (diluted 1:1000; Cell Signaling, Danvers, MA, USA), rat anti-DAT monoclonal antibody (diluted 1:1000, Chemicon), rabbit anti-tyrosine hydroxylase antibody (diluted 1:5000, Chemicon), anti-inducible NO synthase (iNOS) antibody (diluted 1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-neuronal NO synthase (nNOS) antibody (diluted 1:500, Cell Signaling), anti-NF-κB (diluted 1:1000, Santa Cruz Biotechnology), and anti-phospho NF-κB (diluted 1:500, Cell Signaling) for 12 – 24 h at 4°C followed by HRP-conjugated secondary antibody (1: 2000, Santa Cruz Biotechnology) and developed using enhanced chemiluminescent detection methods (ECL kit; Amer sham Pharmacia Biotech, Buckinghamshire, UK).

Repeated injections of METH produced a significant hyperthermia in mice. Pre-treatment with ISL prior to the 1st and 3rd METH injections did not suppress METH-induced hyperthermia (data not shown). Repeated injections of METH caused degeneration of striatal dopaminergic nerve terminals. We assessed the loss of dopaminergic terminals by DAT- and TH-immunoreactivity in the striatum. Representative photomicrographs of DAT staining in the striatum taken 3 days after the last METH injection are shown in Fig. 1A. Reduction of striatal DAT signals was observed 3 days after the last METH injection. The reduction of striatal DAT signals after repeated METH injections was significantly attenuated by pre-treatment with injections of ISL (Fig. 1A). There were no significant changes in striatal DAT signals in the group treated with ISL alone. We confirmed and quantified the expression of DAT and TH by Western blotting. As shown in Fig. 1B, repeated METH injections decreased striatal DAT and TH protein expression by about 80% and 60%, respectively. ISL treatment prior to METH injection attenuated the reduction of DAT and TH protein expression. In contrast, no significant change was detected in the group treated with ISL alone.

Since repeated METH administration is known to induce accumulation of microglia and astrocytes in the striatum (10), we examined the effects of ISL on METH-induced accumulation of activated astrocytes (Fig. 2). Repeated METH injections significantly increased GFAP-positive activated astrocytes in the striatum at
3 days after the last injection (Fig. 2A). However, pre-treatment with ISL inhibited the METH-induced GFAP expression of activated astrocytes in the striatum (Fig. 2A). There were no changes in GFAP-positive astrocytes in the striatum after ISL alone. The result of Western blotting was consistent with that of immunohistochemistry. Repeated METH injections induced a five-fold increase in GFAP protein expression, whereas ISL treatment blocked the increase of GFAP protein expression by METH (Fig. 2B). There was no significant change of GFAP expression by ISL alone.

Next, we examined the effect of ISL on the METH-induced iNOS because ISL has been known to suppress the production of NO through the blockage of iNOS expression in several cell lines (11, 12). As shown in Fig. 3A, repeated METH injections stimulated the expression of iNOS protein. However, pre-treatment with ISL inhibited the increase in METH-induced iNOS protein expression. On the other hand, it is known that the expression of iNOS is regulated by the activation of NF-κB transcriptional factor. Repeated METH injections activated the phosphorylation of NF-κB, whereas ISL treatment prior to METH inhibited the activation of NF-κB (Fig. 3B).

The major findings of the current study are that ISL treatment prior to repeated METH injection significantly prevented the METH-induced reduction of striatal DAT and TH as well as glial cell activation. On the other hand, whether ISL has such an effect against METH-induced neurotoxicity may be arguable because the DAT expression level was slightly higher in the ISL plus METH group than in the normal group and even in the ISL
alone group (Fig. 1B). Thus, ISL might not protect against METH-induced neurotoxicity but potentiates DAT expression. However, this potentiation-like event is not feasible because the difference of DAT expression levels was not statistically significant between the METH plus ISL group and the ISL alone group. Moreover, ISL alone merely increased striatal DAT expression level by 30% compared to the normal group, whereas ISL plus METH increased METH-induced DAT repression by as much as 700% compared to the METH alone group. If a potentiation-like event rather than the protection of neurons occurred, the DAT expression level in the METH plus ISL group should be around 30% higher than that in the METH alone group.

Mechanisms of METH-induced neurotoxicity are not well understood. However, accumulating evidences suggest that METH-induced neurotoxicity involves the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (2). The role of RNS is further supported by the findings that the neurotoxic effects of METH can be inhibited by NOS inhibition (13). ISL has a variety of cellular functions including an antioxidant effect. ISL has been shown to attenuate the lipopolysaccharide (LPS)–induced NO production by the inhibition of the NF-κB signaling pathway (11). ISL was also reported to modulate the expression of several proteins involved in oxidative stress in cocaine-treated rat striatum (9). In the present study, we found that ISL suppressed the expression of iNOS and the activation of NF-κB in METH-treated mouse striatum. Thus, this result suggests that the protective effects of ISL against METH-induced neurotoxicity might be due to its suppressive effects on oxidative stress, which were mediated by the repression of NOS through the blockage of NF-κB signal transduction. In-depth studies are required to clarify its suppressive effect on METH-induced oxidative stress.

Meanwhile, this study showed that pretreatment with ISL had no effect on METH-induced hyperthermia. Increased body temperature has been reported to be involved in METH neurotoxicity. However, reserpine inducing hypothermia does not prevent the neurotoxic effects of METH (14). In addition, IFN-γ completely prevents the neurotoxicity to dopaminergic neurons but does not alter METH-induced hyperthermia (15). These findings suggest that hyperthermia might contribute to, but is not the sole cause of, the neuropathology produced by METH or MDMA. In similar context, the neuro-protective effect of ISL might be independent of its effect on body temperature.

In conclusion, ISL protected against METH-induced dopaminergic neurotoxicity and inhibited glial cell activation as well as NOS expression in the striatum. These results suggest that ISL modulates the METH-induced oxidative stress, suggesting the potency of ISL as a novel therapeutic agent to prevent METH-induced neurotoxicity.

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References

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