1. Introduction

Methamphetamine (METH)-induced neurotoxicity is characterized by a long-lasting depletion of striatal dopamine (DA) as well as damage to striatal dopaminergic nerve terminals. Auto-oxidation of cytosolic free DA and consequent generation of reactive oxygen species (ROS) were implicated in METH-induced neurotoxicity of dopaminergic neurons (1). In addition, previous studies demonstrated involvement of some inflammatory molecular events in METH-induced dopaminergic-specific neurotoxicity, such as induction of cyclooxygenase (COX)-2 in the striatum (2) and activation of microglia (3), because these neurons were protected by use of several nonsteroidal anti-inflammatory drugs (NSAIDs) (3, 4) and minocycline (5), which inhibit COX activity and microglia activation, respectively.

Phytochemicals are chemical compounds that occur naturally in plants; these include polyphenols, flavonoids, and other chemicals. These natural, potent phytochemicals have been shown to exert beneficial, protective effects in cardiovascular diseases, cancer, infections, and neurodegenerative disorders. In addition, these chemicals have been shown to scavenge pathological concentrations of ROS and reactive nitrogen species (RNS) and to chelate transition metal ions (6–9). Therefore, phytochemicals protect dopaminergic neurons and glial cells from damage caused by psychostimulants or neurotoxins. The objective of this review was to evaluate the involvement of glial cells in dopaminergic neuron–specific toxicity and to explore the neuroprotective activity of phytochemicals in terms of anti-inflammatory and antioxidant action.

Keywords: dopaminergic neurotoxicity, psychostimulant, neurotoxin, phytochemical
In this review, we focus on the involvement of glial cells in dopaminergic neuron-specific toxicity and the neuroprotective activity of phytochemicals in terms of anti-inflammatory and antioxidant action.

2. Protective effects of phytochemicals on neurotoxin-induced toxicity

2.1. SFN (1-isothiocyanato-4-methylsulfinylbutane)

The isothiocyanate SFN is an organosulfur compound derived from a glucosinolate precursor that is found in cruciferous vegetables, such as Brussels sprouts, broccoli, cauliflower, and cabbage (10). Chen et al. (11) reported that SFN inhibited METH-induced behavioral changes in mice, such as acute hyper-locomotion and the development of behavioral sensitization. The inhibition, at least in part, of behavioral effects suggests that SFN acts by decreasing extracellular DA levels in the mouse striatum; however, it remains unclear as to whether activation of nuclear factor-erythroid 2-related factor 2 (Nrf2), a master antioxidant transcription factor, accounts for the fact that SFN diminishes acute METH-induced behavioral changes.

Notably, METH-induced neurotoxicity in the mouse striatum, including METH-induced reductions of DA and 3,4-dihydroxyphenylacetic acid levels and DA transporter (DAT) immunoreactivity, was attenuated by both pretreatment and subsequent administration of SFN.

In this regard, it has been reported that METH-induced toxicity at DA nerve endings within the striatum is associated with microglial activation (3). It has been demonstrated that SFN is a potent inhibitor of microglial activation (12). SFN reduces METH-induced neurotoxicity and microglial activation in the mouse striatum. The neurotoxin 6-hydroxydopamine (6-OHDA) has been widely used to produce models of the DA neuronal degeneration of Parkinson’s disease (PD). Tarozzi et al. (13) demonstrated that among cellular antioxidant defenses up-regulated by SFN, the improvement of total glutathione (GSH) levels is crucial to protect the neuroblastoma SH-SY5Y cell line against chemically-induced oxidative damage in terms of impairment of the intracellular redox state and cellular death. Treatment with SFN resulted in significant increases of total GSH levels and GST, GR, and NADPH quinone oxidoreductase-1 (NQO-1) activities, but not GSH-peroxidase, catalase, and superoxide dismutase (SOD) activities. Moreover, the elevation of GSH levels and activities of GSH-transferase (GST) and NQO-1 was correlated to an increase of the resistance of SH-SY5Y cells to toxicity induced by H$_2$O$_2$ or 6-OHDA. The pre-treatment of SH-SY5Y cells with SFN was also shown to prevent various apoptotic events (mitochondrial depolarization, caspase activation, and DNA fragmentation) and necrosis elicited by 6-OHDA. Furthermore, the impairment of antioxidant capacity and ROS formation at the intracellular level after exposure to 6-OHDA was effectively counteracted by pretreatment with SFN. In addition, both the cytoprotective and antioxidant effects of SFN were abolished by inhibition of GSH synthesis, supporting the main role of GSH in the neuroprotective effects exerted by SFN. Morroni et al. (14) reported that the increase in 6-OHDA-induced rotations and deficits in motor coordination were ameliorated significantly by SFN treatment. SFN protected against 6-OHDA-induced apoptosis via blocking DNA fragmentation and caspase-3 activation. In immunohistchemical findings, SFN-treatment protected neurons from neurotoxic effects of 6-OHDA. SFN treatment also increased GSH content and GST and GSH reductase (GR) activities in 6-OHDA-lesioned mice. The neuroprotective effect of SFN may be attributed to the enhancement of GSH levels and its dependent enzymes (GST and GR) and to modulation of the neural survival pathways, such as the extracellular signal-regulated kinase (ERK1/2) pathway, in the mouse brain.

It has been reported that SFN increased Nrf2 protein levels in the mouse basal ganglia and led to up-regulation of the phase II antioxidant enzymes heme oxygenase-1 (HO-1) and NQO-1. In wild-type, but not in Nrf2-knockout, mice, SFN protected against death of nigral dopaminergic neurons induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridene hydrochloride (MPTP) and protected against nigral dopaminergic cell death, astrogliosis, and microgliosis in the MPTP mouse model of PD (15). Moreover, SFN prevented H$_2$O$_2$- or paraquat-induced cytotoxicity in rat striatal cultures by increasing intracellular GSH levels through an elevation in γ-glutamylcysteine synthetase expression induced by activation of the Nrf2-antioxidant response element pathway (16).

In regard to metabolism and tissue distribution of SFN in Nrf2 knockout (Nrf2$^{−/−}$) and wild-type mice, which were given SFN by oral gavage, SFN is metabolized and reaches target tissues including the brain in both wild-type and Nrf2$^{−/−}$ mice (17).

The mechanism of Nrf2 activation by SFN has been demonstrated to involve disruption of Nrf2-Keap1 and is associated with modification of critical Keap1 cysteine residues (18). Therefore, SFN can play an important role in the protection of striatal dopaminergic neurons against toxicity via increasing Nrf2 expression.

2.2. Baicalein (5,6,7-trihydroxy-2-phenyl-chromen-4-one, 5,6,7-trihydroxyflavone)

The flavonoid baicalein is an aglycone of baicalin derived from the root of Scutellaria baicalensis GEORGI.
Baicalin has been utilized in traditional Chinese herbal medicine as an antibacterial, antiviral, and anti-inflammatory agent (8). With respect to the pharmacokinetics and tissue distribution of baicalin in rats using a microdialysis study, baicalin was able to penetrate the blood–brain barrier as well as undergoing hepatobiliary excretion (19). Baicalin shows antioxidant effects and a free radical scavenging effect, which prevent MPTP-induced dopaminergic neurotoxicity through improvement of the resulting abnormal GSH peroxidase activity and prevention of lipid peroxidation (20). Baicalin pretreatment also significantly increased the SOD activity. Considered together, these results suggest that baicalin could inhibit oxidative stress via an increase in the activity of antioxidant enzymes (20).

Takeshima et al. (21) reported that baicalin prevented L-DOPA-induced reduction of the number of viable cells and enhanced protein-bound quinone (quinoprotein) formation in cultured dopaminergic CATH.a cells. Moreover, baicalin prevented the formation of DA semiquinone radicals in an in vitro cell-free system; in addition, long-term treatment with baicalin upregulated intracellular GSH content. The report indicates that baicalin suppressed excess L-DOPA-induced quinone generation by scavenging DA quinone in dopaminergic neurons.

Wu et al. (22) demonstrated a neuroprotective effect of baicalin against METH-induced striatal damage in mice. Pretreatment with lower doses (0.3 – 1.0 mg/kg, i.p.), but not higher doses (3 mg/kg, i.p.), of baicalin significantly attenuated the METH-induced striatal DAT loss in a dose-dependent manner. Moreover, baicalin diminished the METH-induced increase in striatal malondialdehyde content and myeloperoxidase activity. In addition, baicalin diminished ROS production by leukocytes stimulated with METH. Although METH-induced neuronal nitric oxide synthase (nNOS) overexpression was further increased by pretreatment with baicalin, the nNOS level was not altered by baicalin treatment alone. The report suggests that baicalin may attenuate METH-induced DAT loss by inhibiting the neutrophil increase and lipid peroxidation caused by neutrophil-derived ROS in the striatum. Baicalin also has been shown to have an inhibitory effect on xanthine oxidase.

Baicalin reduces the mitochondrial dysfunction, caspase activation, and expression of phospho-c-Jun N-terminal kinase (JNK) associated with 6-OHDA-treatment of SH-SY5Y cells (23). More recently, it has been reported that baicalin prevented loss of nigrostriatal DA neurons and astroglial activation in MPTP-injected PD model mice and downregulated MPTP-induced activations of NF-κB, ERK, and JNK in astrocytes (24).

Taken together with these findings, baicalin may protect dopaminergic neurons from psychostimulants- or neurotoxin-induced neuronal damages by modulation of neuronal or astroglial molecules that are associated with oxidative stress and neuroinflammation.

2.3. EGCG ([(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl]3,4,5-trihydroxybenzoate)

Experimentally, tea (Camellia sinensis) and its constituent polyphenols have been shown to have flavanol antioxidant, iron-chelating, anti-inflammatory, and anti-carcinogenic properties, both in vivo and in vitro (9). EGCG is absorbed from the digestive tract with the intestinal mucosa being most enriched of the organelles after a single oral administration of EGCG in rats. The EGCG levels found in the brain tissue corresponded to 0.0003% – 0.45% of ingested EGCG (25). Pretreatment of mice with either green tea extract or EGCG prevented the MPTP-induced loss of nigral DA neurons that occurs concomitantly with depletion of striatal DA and TH protein levels (26). In addition, EGCG prevented an increase of striatal SOD and catalase activities. The researchers speculated that the neuroprotective effects are not likely to be due to inhibition of MPTP conversion to its active metabolite MPP+ by monoamine oxidase B because both green tea extract and EGCG are very poor inhibitors of this enzyme in vitro. Moreover, the neuroprotective effect of EGCG against MPTP toxicity may involve its structural resemblance to catechol, blocking MPP+ uptake, and to up-regulation of antioxidant enzymes such as SOD and catalase.

Chao et al. (27) reported that a pro-drug, fully acetylated EGCG (pEGCG) with improved stability and bioavailability (which had already been demonstrated in cancer research to reduce and inhibit tumor growth in several tissues), attenuated cell death of SH-SY5Y cells caused by 24-h exposure to 6-OHDA. pEGCG also was effective in reducing caspase-3 activity, but not at all concentrations tested. pEGCG treatment markedly increased 6-OHDA-induced loss of Akt phosphorylation levels. The authors indicated that pEGCG has potential for development as a protective agent for treatment of neurological degenerative disorders such as PD.

Pretreatment with EGCG attenuated SH-SY5Y cell death caused by 24-h exposure to 6-OHDA and also restored the reduced protein kinase C (PKC) and ERK1/2 activities caused by 6-OHDA toxicity. The neuroprotective effect of EGCG on cell survival was abolished by pretreatment with the PKC inhibitor GF109203X. In addition, gene expression analysis revealed that EGCG prevented both the 6-OHDA-induced expression of several mRNAs, such as Bax, Bad, and Mdm2, and the decrease in Bcl-2, Bclw, and Bcl-xL. The authors
suggested that the neuroprotective mechanism of EGCG against oxidative stress-induced cell death includes stimulation of PKC and modulation of cell survival and the cell cycle (28).

Therefore, the activation of PKC by EGCG may play as significant a role in its neuroprotective mechanism of action as does its intrinsic antioxidant capacity.

NO has been proposed to have a role in the inflammatory processes occurring in PD. EGCG prevented an elevation in striatal and substantia nigral nigral inducible NOS (iNOS) in an MPTP mouse model of PD (29). EGCG treatment reduced MPTP-induced neuronal death to less than 50%. The level of iNOS expression in the MPTP group was 20% higher than that in the control group, but iNOS expression in the EGCG-treated group was reduced to the control level.

EGCG is known to directly counteract RNS toxicity by scavenging peroxynitrite and its progenitor, superoxide. It has been reported that there was an apparent positive correlation between the number of hydroxyl groups, particularly of the B-ring, and the anti-radical activity. In addition, the anti-inflammatory activity of EGCG also depends on the inhibition of expression of inflammatory genes such as the one encoding iNOS, mainly through interfering with the activation of pro-inflammatory transcription factors such as NF-κB (6).

3. Role of glial cells in METH-induced neurotoxicity

Recently, accumulating evidence has demonstrated the involvement of glial cells in the METH-induced damage of DA neurons. Neuroinflammation and microglial activation are known to contribute to the degeneration of dopaminergic nerve terminals. Accumulation of activated microglia in the rodent striatum was seen in METH-induced toxicity (3). In addition, in human METH abusers, microgliosis in the brain was detected by positron emission tomography (30) and by immunohistochemistry (31). In microglia activated by various stimuli, NF-κB which promotes COX-2 and iNOS inductions, is activated to cause neuroinflammation and apoptosis. Pretreatment with minocycline, a potent inhibitor of microglial activation, attenuated the reduction in DA, its metabolite, and DAT-immunoreactivity in the striatum after the repeated administration of METH, in a dose-dependent manner (5). Furthermore, the post-treatment and subsequent treatment with minocycline also attenuated METH-induced neurotoxicity. METH administration caused activation of NF-κB and generation of NO, probably in microglia. METH induced COX-2 expression coinciding with microglial activation and DAT reduction in mouse striatum, which were attenuated by co-administration of some NSAIDs, including ketoprofen and indomethacin, but not aspirin (3). However, understanding of the precise involvement of COX-2 in METH-induced neurotoxicity remains controversial. COX-2 null mice were resistant to METH-induced neurotoxicity, whereas COX-2 inhibitors did not protect against METH neurotoxicity. The selective COX-2 inhibitor cerecoxib exacerbates METH-induced DA depletion in rat striatum. These findings suggest that the protective effects of some NSAIDs against METH toxicity is based not only on their COX-inhibiting effects but also on other properties. The reduction of DAT-positive signals and nuclear peroxisome proliferator-activated receptor γ (PPARγ) expression and accumulation of activated microglial cells were dose-dependently attenuated by injections of an NSAID and PPARγ ligand ibuprofen given 30 min prior to each METH injection, but not by either a low or high dose of aspirin, although treatment with either ibuprofen or aspirin significantly blocked METH-induced striatal COX expression (32). Furthermore, treatment with the intrinsic PPARγ ligand 15d-PG J2 also attenuated METH-induced reduction of striatal DAT (32). These results imply that the protective effects of ibuprofen or indomethacin against METH-induced neurotoxicity may be based, in part, on anti-inflammatory PPARγ agonistic activity or inhibiting microglial activation, but not on the COX-inhibiting property. Pretreatment with SFN significantly prevented striatal reduction of DA and DAT and microglial activation induced by repeated METH administration (11). However, some reports showed that minocycline failed to prevent METH-induced neurotoxicity, although it attenuated METH-induced microglial activation (5, 33). Furthermore, pretreatment with edaravone, a radical scavenger, reduced METH-induced striatal dopaminergic neuron degeneration and the activation of astrocytes, but did not affect activation of microglia (34). Therefore, microglial activation per se may not be obligatory for METH neurotoxicity.

Neuroinflammatory cytokines related to microglia are involved in acute METH-induced neurotoxicity (1). The METH-induced DAT reduction and microgliosis in the striatum was ameliorated in interleukin-6 (IL-6)-knockout mice. METH activates NFAT (nuclear factor of activated T cells), a target transcription factor for immunosuppressant, and decreases the peripheral production of IL-2 and interferon-γ (IFN-γ) levels. Furthermore, striatal dopaminergic neurons were protected from METH-induced toxicity by the immunosuppressant FK506. Our previous study demonstrated that intraperitoneal injection of IFN-γ prevented METH-induced reduction of striatal DAT and hyperthermia and that intracerebroventricular injection of IFN-γ markedly prevented the METH neurotoxicity but not hyperthermia.
These findings suggest that peripheral inflammation- or immune reaction–related molecules protect against METH-induced neurotoxicity through intracerebral molecular pathways that are probably unrelated to hyperthermia.

METH is known to promote oxidative stress, including ROS/RNS and DA quinone formation as dopaminergic neuron–specific oxidative stress, via cytosolic DA elevation to cause striatal neurotoxicity (1). These ROS/RNS and DA quinones, and the consequent dopaminergic neuronal damage, are scavenged or ameliorated by GSH and thiol reagents. We previously reported that METH increased DA quinones and NQO-1 coinciding with neurodegeneration and that the Nrf2-inducer butylated hydroxyanisole, which promotes transcription of NQO-1 and GSH-related enzymes, blocked METH-induced elevation of quinoprotein and dopaminergic cell death. Nrf2 deficiency exacerbated METH-induced damage to DA neurons and potentiated activation of both microglia and astrocytes in the striatum (35). Since Nrf2 is dominantly expressed in astrocytes in the brain, astrocytes and their antioxidative system may contribute to protection from neuronal damage caused by METH-induced oxidative stress. Astrocytes also contribute to METH-induced changes in glucose uptake. METH exposure inhibited astroglial glucose uptake in parallel with changes in glucose transporter protein-1, and these changes were stabilized by treatment with acetyl-l-carnitine for enhanced production of ATP from fatty oxidation (36).

4. Conclusions

Potential therapeutic strategies targeting antioxidative molecules in astrocytes, to protect DA neurons from oxidative stress and progressive degeneration induced by psychostimulants or neurotoxins, have been proposed as an important approach for developing an efficient clinical tool against PD progression and amphetamine/stimulant psychosis. Several phytochemicals can have important influences on health status and disease states and thus could be targeting antioxidative molecules in astrocytes to protect DA neurons from oxidative stress and progressive neurodegeneration (Table 1). The isothiocyanate SFN has important implications for protection of dopaminergic neurons in the striatum against toxicity by increasing Nrf2 expression. The flavonoid baicalein could protect DA neurons from oxidative stress through increases in the activity of antioxidant enzymes. Baicalein may provide protection for DA neurons by inhibiting the modulation of astroglial molecules that are associated with oxidative stress and neuroinflammation caused by psychostimulants or toxins. The flavanol antioxidant EGCG has neuroprotective effects; its mechanism of action is connected to the activation of PKC, which may play as significant a role in neuroprotection as does its intrinsic antioxidant capacity.

It is still unclear whether cellular or molecular events in glial cells are involved in the neuroprotective effects of phytochemicals reviewed in this article. Further

Table 1. Effects of phytochemicals on psychostimulant- or neurotoxin-induced neurotoxicity

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