Protective effects of atypical antipsychotic drugs against MPP⁺-induced oxidative stress in PC12 cells

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Abstract
Recent studies have suggested that some atypical antipsychotic drugs may have protective properties against oxidative stress. To confirm these findings, we investigated the protective effects of atypical antipsychotic drugs such as olanzapine, aripiprazole, and ziprasidone on oxidative stress induced by the N-methyl-4-phenylpyridinium (MPP⁺) ion in PC12 cells. Haloperidol, a typical antipsychotic drug, was used for comparison. We determined the antioxidant effects of atypical antipsychotic drugs using a number of measures, including cell viability, the formation of reactive oxygen species (ROS), superoxide dismutase (SOD) activity and Bax levels. MPP⁺ treatment induced significant loss of cell viability, the formation of ROS, reduction of SOD activity, and up-regulation of Bax expression. However, olanzapine, aripiprazole, and ziprasidone reversed these effects caused by MPP⁺ treatment, but ziprasidone did not influence cell viability. In contrast, haloperidol did not affect all these effects. Moreover, haloperidol strongly increased the expression of Bax under MPP⁺-free conditions. Olanzapine, aripiprazole, and ziprasidone, but not haloperidol, may exert antioxidant effects through modulating ROS levels, SOD activity, and Bax expression to provide protective effects against MPP⁺-induced oxidative stress in PC12 cells. These results suggest that some atypical antipsychotic drugs have a useful therapeutic effect by reducing oxidative stress in schizophrenic patients.

1. Introduction
The etiology of schizophrenia remains unclear. A good deal of evidence suggests that oxidative stress may play an important role in the pathophysiology of schizophrenia (Smythies, 1997; Fenton et al., 2000). Oxidative stress occurs due to an imbalance between levels of free radical production and the efficiency of the antioxidant enzyme system to neutralize and eliminate reactive oxygen species (ROS). Excessive accumulation of ROS and inefficiency of the antioxidant enzyme system can damage DNA and induce lipid peroxidation and protein modification, causing cellular dysfunction and even apoptosis (Lohr and Browning, 1995; Stadtman, 2004). In the brain, the main antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). SOD is the most important enzyme for the detoxification of ROS and protection against oxidative stress, and it can thus help prevent neuronal cells from apoptosis (Greenlund et al., 1995).

Many researchers have demonstrated abnormal antioxidant enzyme activity in schizophrenic patients, including in the activity of SOD, CAT, and GSH-Px. For example, schizophrenic patients have been found to have increased levels of SOD and GSH-Px activity (Kuloglu et al., 2002; Dakhale et al., 2004). Moreover, lipid peroxidation was increased in the plasma (McCreadie et al., 1995; Mahadik et al., 1998), red blood cells (Herken et al., 2001), and cerebrospinal fluid of schizophrenic patients (Lohr et al., 1990). However, other studies have found low red blood cell SOD activity (Mukerjee et al., 1996) and unchanged GSH-Px activity (Yao et al., 1998). These controversial results may be due to treatment with antipsychotic drugs. It has been conjectured that typical antipsychotic drugs such as haloperidol may have pro-oxidant properties, whereas some atypical antipsychotic drugs may not induce oxidative stress (Singh et al., 2008; Parikh et al., 2002). Atypical antipsychotic drugs show better therapeutic efficacy for negative symptoms and cognitive deficits and have less propensity to induce extrapyramidal symptoms (EPS) than do typical antipsychotics drugs (Meltzer, 1995; Andreasen, 1994). Although these differences may contribute to some of the

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different therapeutic effect and side effect profiles of antipsychotic drugs, the mechanisms of their action have yet to be elucidated.

In vitro studies have shown that atypical antipsychotic drugs offer a protective function through the upregulation of SOD in apoptotic models using hydrogen peroxide and serum withdrawal, which cause oxidative stress in PC12 cells (Wei et al., 2003a,b; Bai et al., 2002). On the other hand, haloperidol has been reported to increase lipid peroxidation in schizophrenic patients with tardive dyskinesia (Zhang et al., 2007), to decrease SOD, CAT, and GSH-Px in rat brains (Naidu et al., 2003), and to cause apoptotic cell death (Behl et al., 1995; Noh et al., 2000). Moreover, a preclinical study by Pillai et al. (2007) showed that post-treatment with atypical antipsychotic drugs such as olanzapine, clozapine and risperidone for 90 days after 90 days of haloperidol treatment restored to normal the haloperidol-induced losses in manganese-SOD (MnSOD) and copper-SOD (CuSOD) activities and increases in lipid peroxidation products.

In the present study, we used 1-methyl-4-phenylpyridinium (MPP+) ion, a dopaminergic neurotoxin, to produce oxidative stress. MPP+, an active metabolite of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), causes loss of dopaminergic neurons by its accumulation via dopamine transporter uptake (Pilb, et al., 1993) and free radical generation (Tipton and Singer, 1993). Therefore, the MPP+ toxicity model may have relevance as a pathogenesis for schizophrenia related to not only oxidative stress (Smythies, 1997; Heinz and Schlagenhaus, 2010). This agent was reported to induce apoptosis in cultured cerebellar granule neurons (Dipasquale et al., 1991), rat pheochromocytoma (PC12) cells (Mutoh et al., 1994), and nigral–striatal cocultures (Mochizuki et al., 1994). Neuronal cell death due to MPP+ is mediated by opening of the mitochondrial permeability transition pore, releases of Ca2+ and cytochrome C from the mitochondrial permeability and activation of caspases (Cassarino et al., 1999; Lotharius et al., 1999). In addition, the pro-apoptotic protein Bax affects mitochondrial permeability and induces cytochrome C release, subsequently leading to apoptosis (Crompton, 2000).

In this study, we investigated whether the atypical antipsychotic drugs, olanzapine, ziprasidone, and aripiprazole, have antioxidant effects on MPP+-induced oxidative stress in PC12 cells. It is now well established that undifferentiated PC12 cells which synthesize, store, and secrete dopamine (Schubert and Klier, 1977) have been used in studies on neuroprotective effects of atypical antipsychotic drugs (Qing et al., 2003; Wang et al., 2005; Wei et al., 2003a,b). We used haloperidol, a prototype agent for typical antipsychotic drugs, for comparison with atypical antipsychotic drugs. In the present study, to determine the protective effects of atypical antipsychotic drugs against oxidative stress, we evaluated whether the atypical antipsychotic drugs, olanzapine, ziprasidone, and aripiprazole, prevent viability loss, ROS elevation, and SOD activity reduction caused by MPP+ in PC12 cells. We also demonstrated the alteration in the level of Bax leading to mitochondrial damage.

2. Materials and methods

2.1. Materials

Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum (FBS), and antibiotic–antimycotic were purchased from GibCO BRL (Grand Island, NY, USA), MPP+, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), dichloro-fluorescin diacetate (DCFH-DA), and haloperidol from Sigma (St. Louis, MO, USA), olanzapine from Lilly Research (Indianapolis, IN, USA), aripiprazole from Otsuka Pharmaceutical (Tokushima, Japan), ziprasidone from Pfizer (New York, NY, USA), and SOD enzyme assay kit from Cayman Chemical (Ann Arbor, MI, USA). All other chemicals were purchased from commercial sources.

2.2. Cell culture

The rat pheochromocytoma cell line PC12 was cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 U/ml streptomycin, and 25 µg/ml amphotericin B. The cell line was typically grown as undifferentiated cells in a 100-mm culture dish at 37°C in 5% CO2. When the cells were 70% confluent, they were harvested and dispersed. The dispersed cells were then cultured for 48–72 h with antipsychotic drugs in the presence or absence of MPP+. The culture medium was changed every 2–3 days.

2.3. Drug treatment

20 mM of olanzapine and 10 mM of the other three antipsychotic drugs were completely dissolved in phosphate-buffered saline (PBS) and dimethyl sulfoxide (DMSO), respectively. The solutions were diluted to various concentrations with DMEM medium before use. Aripiprazole, ziprasidone and haloperidol were diluted to final concentration of 0.5% DMSO. To investigate the protective effect of antipsychotic drugs, PC12 cells were cultured for 48 or 72 h with olanzapine (1, 10, 50, and 100 µM), aripiprazole (10, 20, and 50 µM), ziprasidone (10, 20, and 50 µM), and haloperidol (10, 20, and 50 µM) in the presence or absence of 250 µM or 1 mM MPP+. Control cells were cultured without MPP+ and antipsychotics.

2.4. Cell viability assay

Cell viability was measured using the MTT assay. PC 12 cells were seeded on 96-well plates at a density of 4 × 103 cells/well and then cultured for 48 h in media with or without 250 µM MPP+ with various concentrations of antipsychotic drugs. Cell viability was then determined using colorimetric measurement of the reduction product of MTT. Then, the culture medium was removed, 100 µl of a DMSO solution was added to each well, and the plates were incubated at 37°C for 4 h to dissolve the formazan that had formed. Reduced MTT was measured on an ELISA reader (SLT Spectra; SLT Instruments, Salzburg, Austria) at a wavelength of 570 nm. Values for each treatment group are expressed as a percentage of the control.

2.5. ROS activity

To measure ROS production in the PC12 cells produced by MPP+ treatment, we used a DCFH–DA assay, described as follows. DCFH-DA is a fluorescent dye that crosses the cell membrane and is enzymatically hydrolyzed by intracellular esterases to nonfluorescent DCFH. The cells were plated at a density of 4 × 103 cells per 6-well dish. The cells were then treated with various concentrations of antipsychotic drugs in media with or without 1 mM MPP+ for 48 h. The cells were incubated with DCFH–DA at a final concentration of 100 µM in PBS for 1 h at 37°C, and then washed three times with PBS. ROS levels were measured using a FACScan (Becton Dickinson, San Jose, CA, USA) with excitation and emission wavelengths of 475 and 525 nm, respectively. For each analysis, 10,000 events were recorded. The value for each treatment group was converted to the percentage of the control value.

2.6. SOD enzyme activity

The cells were plated at a density of 4 × 105 cells per 6-well dish. The cells were then treated with various concentrations of antipsychotic drugs in media with or without 1 mM MPP+ for 48 h. For whole cell lysates, the cells were washed twice with
ice cold PBS. Lysis buffer [20 mM Tris–HCl, 137 mM NaCl, 10% glycerol, 1% Nonidet p-40, 0.1% sodium dodecyl sulfate (SDS), 0.5% sodium deoxycholate, 2 mM EDTA, 1 tablet complete protease inhibitor (Roche, Laval, Quebec, Canada), 20 mM NaF, and 1 mM Na3VO4] was added, followed by centrifugation (1000 × g, 20 min, 4 °C). Equal 10-μl lysate aliquots were used for determination of SOD enzyme activity as per the manufacturer’s instructions. The SOD assay was measured in all three types of SOD (Cu/Zn-, Mn-, and Fe-SOD). SOD activity was measured using an ELISA reader at a wavelength of 450 nm. Values for each treatment group are expressed as a percentage of the control value.

2.7. Western blot analysis

The cells were plated at a density of 4 × 10^5 cells per 6-well dish. The cells were treated with various concentrations of antipsychotic drugs in media with or without 1 mM MPP+ for 72 h. For whole cell lysates, the cells were washed twice with ice cold PBS. The whole-cell lysates were prepared as follows. Cells were washed twice with ice cold PBS. Lysis buffer was then added, followed by centrifugation (1000 × g, 10 min, 4 °C). Equal amounts of protein (20 μg) from the cell extracts in each treatment condition were separated using 15% sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, and then transferred electrophoretically onto polyvinylidene fluoride (PVDF). The blots were blocked by incubation in 5% (w/v) no-fat milk in PBS with 0.1% Tween 20 (PBS-T) for 1 h. After incubation with a primary antibody [anti-Bax (Santa Cruz, CA, USA) 1:1000 and anti-α-tubulin (Sigma, MO, USA) 1:2000] in PBS-T at 4 °C overnight, the membranes were washed three times in PBS-T for 5 min. Subsequently, the membranes were incubated for 1 h in PBS-T containing the appropriate horseradish peroxidase-conjugated secondary antibody [anti-mouse IgG (Amersham, Buckinghamshire, UK) 1:2000 and 1:10,000, respectively]. The immunoreactive bands were visualized and quantified using ECL + Western blotting reagents, with chemiluminescence detected by Las-3000 Image Reader software (Fuji Film, Tokyo, Japan). Protein levels were normalized to the housekeeping protein α-tubulin to adjust for variability of protein loading and expressed as a percentage of the vehicle control (deemed to be 100%).

2.8. Statistical analysis

All statistical analyses were carried out using repeated measures one-way ANOVAs followed by Scheffe’s post hoc tests. p-Values ≤ 0.05 were deemed statistically significant.

3. Results

3.1. Effects of antipsychotic drugs on cell viability

To assess the effects of antipsychotic drugs on MPP+-induced cytotoxicity, we analyzed cell viability using the MTT assay. Exposure to 250 μM MPP+ for 48 h reduced cell viability to about 34% compared with control cells (p < 0.01, Fig. 1). Olanzapine up to a dose of 100 μM significantly reduced cell loss induced by MPP+ (p < 0.05 or p < 0.01, Fig. 1A). In addition, treatment with olanzapine at 100 μM increased cell viability by 17% under the MPP+-free condition compared with control levels. Aripiprazole at the dose range of 10–50 μM significantly reduced MPP+-induced cell death by 10–14% (p < 0.01, Fig. 1B). However, no changes were observed in cultures treated with ziprasidone (10–50 μM) or haloperidol (10–50 μM) (Fig. 1C and D).

Fig. 1. Effects of antipsychotic drugs on the cell viability in PC12 cells. Cells were treated with different doses of antipsychotic drugs for 48 h with (+MPP+) or without (−MPP+) 250 μM MPP+, after which the MTT assay was performed. (A) Olanzapine; (B) aripiprazole; (C) ziprasidone; (D) haloperidol. Values are expressed as a percentage of the control value (−MPP+, no drug treatment) and represent means ± S.E.M. from the three independent experiments. **p < 0.01 vs. untreated control; + p < 0.05 vs. MPP+ treated only; ++ p < 0.01 vs. MPP+ treated only; "p < 0.01 vs. untreated control; ’p < 0.05 vs. MPP+ treated only."
Fig. 2. Effects of antipsychotic drugs on ROS formation in PC12 cells. Cells were treated with different doses of antipsychotic drugs for 48 h with (+MPP+) or without (−MPP+) 1 mM MPP+. The fluorescence intensity of DCF was measured as described in Section 2. (A) Olanzapine; (B) aripiprazole; (C) ziprasidone; (D) haloperidol. Values are expressed as a percentage of the control value (−MPP+, no drug treatment) and represent means ± S.E.M. from the three independent experiments. **p < 0.01 vs. untreated control; "p < 0.05 vs. MPP+ treated only, ++p < 0.01 vs. MPP+ treated only.

3.2. Effect of antipsychotic drugs on ROS formation and SOD activity

To examine whether the effects of atypical antipsychotic drugs on MPP+-induced toxicity are mediated through antioxidant actions, intracellular ROS levels and SOD activity were measured. Following 1 mM MPP+ treatment for 48 h, ROS levels were greatly increased from 100% (control level) to 205% (p < 0.01, Fig. 2), indicating that MPP+ enhanced ROS generation in PC12 cells. However, atypical antipsychotic drugs, olanzapine (10–100 μM), aripiprazole (10–50 μM), and ziprasidone (10–50 μM) effectively reduced ROS formation, and the suppressing effect increased dose-dependently (p < 0.05 or p < 0.01, Fig. 2A–C). On the other hand, no change in the MPP+-induced increase of ROS levels was observed in haloperidol (10–50 μM)-treated cells (Fig. 2D).

The activities of SOD decreased to 34% after MPP+ treatment (p < 0.01, Fig. 3), suggesting that the cytotoxic effect of MPP+ may be mediated by oxidative stress in PC12 cells. Olanzapine (10–100 μM) and aripiprazole (10–50 μM) strongly prevented the MPP+-induced decrease in SOD activity in a dose-dependent manner (p < 0.05 or p < 0.01, Fig. 3A and B). Moreover, treatment with olanzapine at 100 μM significantly increased SOD activity by 15% under MPP+–free conditions (p < 0.05), Ziprasidone showed an inhibitory effect at 10 and 20 μM, but not at 50 μM (p < 0.01, Fig. 3C). However, no significant increase was observed in cells treated with 10–50 μM haloperidol in PC12 cells with or without MPP+ (Fig. 3D).

3.3. Effects of antipsychotic drugs on levels of Bax

We also investigated whether antipsychotic drugs had any effect on expression of the proapoptotic protein Bax under oxidative stress conditions caused by MPP+. As shown in Fig. 4, Bax protein expression significantly increased in 1 mM MPP+-treated cells compared with control conditions (83%, p < 0.01). However, a dose–response profile with olanzapine (10–100 μM) revealed that the level of Bax expression completely decreased at 10 μM, and it was consistent up to 100 μM dose (data not shown). To investigate concentration-dependent decrease, we tested at the concentration range from 1 to 10 μM. Olanzapine treatment (1–10 μM) dose-dependently decreased the level of Bax expression almost to control values (p < 0.05, Fig. 4A). Aripiprazole and ziprasidone at the concentration range of 10–50 μM dose-dependently attenuated the MPP+-induced increase in Bax expression (p < 0.05 or p < 0.01, Fig. 4B and C). Treatment with haloperidol (10–50 μM) selectively and significantly increased Bax expression in the MPP+–free condition (p < 0.05, Fig. 4D), but the level of Bax expression was not affected by adding MPP+ treatment.

4. Discussion

The present study assessed the protective effects of atypical antipsychotic drugs against the cytotoxicity of MPP+ on PC12 cells by determining the cell viability, ROS formation, SOD activity, and Bax expression. Treatment with the atypical antipsychotic drugs, olanzapine (10–100 μM), aripiprazole (10–50 μM), and ziprasidone (10–50 μM), effectively prevented PC12 cells from MPP+-induced oxidative stress, although cell viability was unchanged following ziprasidone treatment. Moreover, olanzapine alone showed the protective effect at 100 μM under the MPP+–free condition. On the other hand, no significant changes in these effects were found in haloperidol (10–50 μM)-treated cells. Haloperidol treatment even elevated Bax levels under the MPP+–free condition.

MPP+ inhibits complex I of the mitochondrial electron transport chain and causes ROS generation (Gluck et al., 1994; Lee et al.,...
1 mM MPP+. SOD activity was assessed as described in Section 2. (A) Olanzapine; (B) aripiprazole; (C) ziprasidone; (D) haloperidol. Values are expressed as a percentage of the control value (−MPP+, no drug treatment) and represent means ± S.E.M. from the three independent experiments. *p < 0.05 vs. untreated control, **p < 0.01 vs. untreated control; †p < 0.05 vs. MPP+ treated only, ‡p < 0.01 vs. MPP+ treated only.

Fig. 3. Effects of antipsychotic drugs on SOD activity in PC12 cells. Cells were treated with different doses of antipsychotic drugs for 48 h with (+MPP+) or without (−MPP+) 1 mM MPP+. SOD activity was assessed as described in Section 2. (A) Olanzapine; (B) aripiprazole; (C) ziprasidone; (D) haloperidol. Values are expressed as a percentage of the control value (−MPP+, no drug treatment) and represent means ± S.E.M. from the three independent experiments. *p < 0.05 vs. untreated control, **p < 0.01 vs. untreated control; †p < 0.05 vs. MPP+ treated only, ‡p < 0.01 vs. MPP+ treated only.

In turn, ROS production is implicated in the initiation of apoptosis (Fall and Bennett, 1999). Cell death induced by MPP+ also manifests as the hallmark feature of apoptosis. The present study showed that MPP+ treatment attenuated cell viability and induced a large increase of ROS production, reduced SOD activity, and the overexpression of Bax in PC12 cells.

Similar results have been reported in a study in which PC12 cells were exposed to MPP+, suggesting that atypical antipsychotic drugs such as clozapine, olanzapine, quetiapine, and risperidone have protective effects (Qing et al., 2003). In that study, it was found that exposure of PC12 cells to 50 μM MPP+ for 24 h resulted in a 35–45% loss of cells, and clozapine (25–50 μM), olanzapine (50–200 μM), quetiapine (10–100 μM), and risperidone (10–50 μM) reduced the MPP+-induced cell loss, whereas haloperidol (10–100 μM) had a cytotoxic effect. This is not consistent with the findings of the present experiment, which found that treatment of 250 μM MPP+ for 48 h reduced cell viability to about 30–40%, and treatment of haloperidol did not affect cell viability. These discrepancies might be attributed to the different paradigms examined in the previous and present studies. In the previous study, PC12 cells were pretreated for 24 h with different doses of antipsychotics and then cultured in the presence of 50 μM MPP+ for another 24 h, whereas in the present study, cells were treated with the drugs and MPP+ simultaneously for 48 h. Moreover, the cells were plated with a density of 1 × 10⁵ cells/well in the previous study, but with a density of 4 × 10⁴ cells/well in the present study.

Previous studies reported that olanzapine, quetiapine, clozapine, and risperidone completely prevented hydrogen peroxide-, serum withdrawal-, β-amyloid peptide-, or MPP+ (50 μM)-induced changes in activities of the key antioxidant enzyme SOD, CAT, and GSH-Px in PC12 cells, whereas haloperidol had no such effects (Wei et al., 2003a; Bai et al., 2002; Qing et al., 2003; Wang et al., 2005). In particular, some studies showed, unlike our findings with MPP+, that β-amyloid peptide and MPP+ increased SOD activity and SOD mRNA expression, respectively, suggesting a compensatory action of cells to maintain cellular redox status (Qing et al., 2003; Wang et al., 2005).

The present study demonstrated for the first time that aripiprazole and ziprasidone protect PC12 cells through reduction of ROS, elevation of SOD activity, and reduction of Bax level from oxidative stress induced by MPP+. In turn, the effects were not observed with drug treatment alone under the MPP+-free condition. According to the recent study mentioned above, aripiprazole prevented chronic mild stress–induced decrease in GSH-Px (Eren et al., 2007) in rat cortices and the glutamate cytotoxicity–induced loss in dopaminergic neurons in rat mesencephalic cultures (Matsuo et al., 2010). In addition, chronic administration of ziprasidone attenuated the immobilization stress–induced decrease in the levels of brain–derived neurotrophic factor (BDNF) in the rat hippocampus and neocortex (Park et al., 2009a). Taken together, these findings suggest that aripiprazole and ziprasidone may only play a protective role under neuronal toxic insult. However, ziprasidone did not affect the reduction of cell viability caused by MPP+ treatment. Although this drug fails to promote cell survival, it may selectively enhance the neurite outgrowth in vitro. In primary hippocampal cultures, ziprasidone significantly increased the number of dendrites, as well as dendrites length (Y.H.K. and S.W.P., unpublished observation). Upregulation of BDNF contributing to neuronal survival, axonal branching, dendrite arborization and synaptogenesis (Lessmann et al., 2003; Ji et al., 2005) could conceivably be responsible for the positive effect of ziprasidone on neurite outgrowth, which may explain the positive effects on antioxidant effects.

In the case of olanzapine, its neuroprotective effects are well known (Gama et al., 2007; Kurosawa et al., 2007; Lieberman et al., 2007; Wakada et al., 2002). In contrast, haloperidol lacks this effect and may even cause oxidative stress, resulting in apoptotic cell death (Behl et al., 1995; Noh et al., 2000). In the present study,
olanzapine up to 100 μM increased cell viability and SOD activity with or without MPP+ treatment. Some studies have reported differential effects of typical and atypical antipsychotic agents in the activity of antioxidant enzymes in the rat brain or in schizophrenic patients. In one rat brain study, chronic haloperidol treatment significantly decreased MnSOD, CuSOD, and CAT activity, with a parallel marked increase in hydroxyalkenals (HAEs), a marker of lipid peroxidation, whereas olanzapine, risperidone, and clozapine treatment did not produce any alteration in the levels of antioxidant enzymes or HAEs (Parikh et al., 2003). Some clinical studies have found that olanzapine treatment for 2 months significantly increased serum total antioxidant status levels (Al-Chalabi et al., 2009) and that haloperidol caused more oxidative stress along with a significant reduction of antioxidant parameters including SOD activity (Singh et al., 2008). In contrast, Zhang et al. (2006) detected no significant alterations of antioxidant enzymes or lipid peroxidation in schizophrenic patients treated with typical and atypical antipsychotic drugs for at least 12 months. Thus, the regulation of antioxidant enzyme activity by antipsychotic drugs may be dependent on the class of antipsychotic drug and the period of treatment. Some evidence also suggests that neurotrophic factors can regulate the activity of the antioxidant enzymes that provide a part of the neuroprotective mechanisms associated with neurotrophic factors (Mattson et al., 1995). We have recently shown that aripiprazole, but not haloperidol, may exert a protective effect via signaling systems associated with BDNF including up-regulation of anti-apoptotic protein Bcl-2 in human neuroblastoma SH-SYSY cells (Park et al., 2009b), and olanzapine also markedly increased the activity of the BDNF gene promoter and the levels of BDNF protein through cAMP response element (CRE)-binding protein (CREB)-mediated gene transcription (Lee et al., 2010). Therefore, it is possible that atypical antipsychotics might elevate SOD activity by up-regulating BDNF levels.

In a postmortem brain study, levels of apoptotic regulatory proteins, Bax and Bcl-2, were altered in temporal cortices of patients with schizophrenia compared with controls (Jarskog et al., 2004). The current study showed that although Bcl-2 was unchanged following treatment (data not shown), 1 mM MPP+ treatment strongly increased Bax levels. Bax protein plays key roles in the effects of ROS on the mitochondrial apoptotic pathway (Kirkland et al., 2002) in that it increases mitochondrial ROS production and promotes apoptosis. In contrast, Bcl-2 stabilizes mitochondrial membrane permeability and prevents the production of ROS from mitochondria, thus inhibiting apoptosis (Lud Cadet et al., 2000). The Bax/Bcl-2 ratio appears more important than Bax and Bcl-2 alone levels in determining apoptotic fate; high Bax/Bcl-2 ratio leads to cell death (Cory and Adams, 2002). Thus, additional study measuring Bax/Bcl-2 ratio is needed to current effort in order to strong evidence on overall apoptotic status.

Now we have found that treatment with haloperidol, but none of the atypical drugs tested, elevated Bax levels under normal conditions, indicating that haloperidol may increase neurotoxicity. Furthermore, it may exacerbate the oxidative stress resulting from increased oxidative dopamine metabolism due to a potent dopamine D2 receptor blockade and increased free radical metabolite production by the drug itself (Mahadik and Mukherjee, 1996; Parikh et al., 2003). It has been suggested that this haloperidol-induced oxidative stress may underlie the pathogenesis of tardive...
dyskinesia in patients with schizophrenia (Lohr et al., 1990). Therefore, the different effects of atypical and typical antipsychotic drugs in the pathogenesis of tardive dyskinesia may reflect differential regulation of Bax protein.

We cannot draw conclusions about the clinical effects of olanzapine, aripiprazole, ziprasidone and haloperidol from our in vitro data, because it is not clear whether these drugs reach concentration in the clinical situation that are sufficient to exert antioxidant effects discussed here. Although the doses of these drugs were generally higher than the normal doses observed in brain tissue, such high doses are routinely used in in vitro studies. These discrepancies might be attributed to the difference between complex brain structures and cell line. In our preliminary experiment, none of antipsychotic drugs used in the present study produced any antioxidant effects in less than 10 μM (except Bax level of olanzapine), and aripiprazole, ziprasidone and haloperidol used at doses over 70 μM increased the amount of nonviable cells by 30–40%. In addition, the ranges of 10–100 μM for olanzapine and 10–50 μM for the other three antipsychotic drugs were based on the previous studies using cytotoxicity models in PC12 cells (Qing et al., 2003; Wang et al., 2005; Wei et al., 2003a,b).

In summary, treatment with olanzapine, aripiprazole, and ziprasidone has been shown to afford neuroprotection as a result of their antioxidant effects, and this was confirmed by our data showing that these drugs were highly effective in the prevention of cell death and reactive oxygen species formation, the reduction of SOD activity, and the elevation of Bax levels induced by MPP+ in PC12 cells. On the other hand, haloperidol may be neurotoxic. Our data suggest that some atypical antipsychotic drugs may have different antioxidant effects compared to haloperidol. These results also suggest that some atypical antipsychotics may have a potentially useful therapeutic effect by reducing oxidative stress in schizophrenia patients. Further studies are needed to elucidate the underpinnings of the anti-oxidant effects of atypical antipsychotic drugs.

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