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Antipsychotics promote the differentiation of oligodendrocyte progenitor cells by regulating oligodendrocyte lineage transcription factors 1 and 2

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ABSTRACT

Aims: Oligodendrocyte/myelin abnormalities may be an important component of the pathogenesis found in schizophrenia. The aim of this current study was to examine the possible effects of the antipsychotic drugs (APDs) haloperidol (HAL), olanzapine (OLA), and quetiapine (QUE) on the development of oligodendroglial lineage cells.

Main methods: CG4 cells, an oligodendrocyte progenitor cell line, were treated with various concentrations of HAL, OLA, or QUE for specific periods. The proliferation and differentiation of the CG4 cells were measured. The regulation of CG4 cell differentiation by oligodendrocyte lineage transcription factors 1 and 2 (Olig1 and Olig2) was examined.

Key findings: The APDs used in this study had no effect on the proliferation of CG4 cells. The APDs elevated the expression of 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP), a specific marker of oligodendrocytes, and promoted the CG4 cells to differentiate into CNP positive oligodendrocytes. QUE and OLA increased the expression of both Olig1 and Olig2 whereas HAL only increased the expression of Olig2.

Significance: Our findings suggest that oligodendrocyte development is a target of HAL, OLA, and QUE and provide further evidence of the important role of oligodendrocytes in the pathophysiology and treatment of schizophrenia. They also indicate that the expression level of oligodendrocyte/myelin-related genes could be profoundly affected by APDs, which should be considered in future studies aiming to measure the oligodendrocyte/myelin-related gene expressions in schizophrenia patients.

Introduction

Antipsychotic drugs (APDs) are the first-line pharmacological treatments for schizophrenia. Recent human studies have found that myelin integrity could be improved by APD treatment in schizophrenia patients (Bartzokis et al., 2007, 2009; Garver et al., 2008). Moreover, in animal studies, APDs such as clozapine, haloperidol (HAL), and quetiapine (QUE) effectively improved behavior in cuprizone-fed mice and ameliorated cuprizone-induced demyelination and oligodendrocyte loss (Xiao et al., 2008; Xu et al., 2010; Zhang et al., 2008). QUE has been found to facilitate the proliferation of cultured neural progenitor cells (NPCs) and differentiation into oligodendrocytes (Xiao et al., 2008). These studies indicate that regulation of oligodendrocyte development and function may be a novel target for APDs.

Oligodendrocytes form the myelin sheath, the plasma membrane extension that wraps around axons in the central nervous system (Sherman and Brophy, 2005). Myelin is the primary constituent of the white matter tracts that form the functional and anatomical connections between various brain regions (Davis et al., 2003). Oligodendrocyte dysfunction influences conduction of action potentials and brain connectivity and has long been considered a vital element in the pathogenesis of schizophrenia (Davis et al., 2003; Hyde et al., 1992).

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Oligodendrocyte development is orchestrated by serial stage marker expressions. The expression of 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP), an enzyme specifically expressed in oligodendrocytes, has been used as a marker of myelin-forming cells (Bifulco et al., 2002; Vogel et al., 1998) and has been found to be altered in the brains of schizophrenia patients (Barley et al., 2009; Dracheva et al., 2006; Flynn et al., 2003; McCullumsmith et al., 2007). Oligodendrocyte lineage transcription factors 1 and 2 (Olig1 and Olig2) are indispensable to the development of oligodendrocytes. Olig1 is critical for the maturation of oligodendrocyte progenitor cells (OPCs) by promoting oligodendrocyte differentiation and is necessary for remyelination (Arnett et al., 2004; Burton, 2005; Lu et al., 2001). Olig2 is necessary for the genesis of oligodendrocytes and myelination (Gokhan et al., 2005; Ligon et al., 2006; Takebayashi et al., 2002). Importantly, Olig2 mRNA has been found to be reduced in the brains of schizophrenia patients (Tkachev et al., 2003). Significant associations between Olig2 gene variations and schizophrenia have been reported in the UK and Chinese populations (Georgieva et al., 2006; Huang et al., 2008). These studies indicate that APDs may have an effect on oligodendrocyte development by regulating the transcription process.

In this study we investigated the effects of APDs including HAL, olanzapine (OLA), and QUE on the development of oligodendroglial lineage cells, using the well-established rat oligodendrocyte progenitor cell line CG4. The potential capacity of APDs to increase the proliferation of CG4 cells was estimated using a cell counting kit-8 (CCK-8) assay. The differentiation of the cell line into oligodendrocytes was assessed by evaluating the levels of CNP expression. The expression of Olig1 and Olig2 in CG4 cells was also examined because they regulate the key stages of early oligodendrocyte development (Ligon et al., 2006).

Material and methods

Antipsychotics

QUE and OLA were provided by AstraZeneca (Wilmington, DE) and Lilly Research Lab (Indianapolis, IN), respectively. HAL was purchased from Sigma-Aldrich (St Louis, MO). All the APDs were dissolved in dimethyl sulfoxide.

CG4 cell culture

CG4 cells continue to proliferate in the continuous presence of B104-conditioned medium for at least 30 passages. When exposed to the appropriate inducing conditions, these cells are able to differentiate into either oligodendrocytes or type 2-astrocytes (Louis et al., 1992). The CG4 cell line was propagated or proliferated on poly-D-lysine (PDL) coated 100-mm dishes with a growth medium containing 70% Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 (1:1), 30% conditioned medium from the B104 neuroblastoma cell line supplemented with N2 supplement (Invitrogen, Burlington, ON, Canada), transferrin (Invitrogen, Burlington, ON, Canada) and Insulin–Transferrin–Selenium-A (Invitrogen, Burlington, ON, Canada). The CG4 cells differentiated after withdrawal of B104-conditioned medium. Two percent fetal bovine serum was added into the differentiation medium to increase the survival of cells. Cells were cultured at 37 °C with 5% CO_2/95% air.

B104 neuroblastoma cell culture

B104 neuroblastoma cells were grown in T75 flasks with DMEM/F12 (1:1) medium containing 10% (v/v) fetal bovine serum at 37 °C with 5% CO_2/95% air. When cells reached 70% confluence, the medium was replaced with DMEM/F12 (1:1) containing N2 supplement, in which the cells were cultured for 3 days. This conditioned medium was aspirated, filtered, and frozen for further use.

CCK-8 assay

The number of viable cells was estimated using the CCK-8 assay (Dojindo Molecular Technologies, Burlington, ON, Canada), which provides effective and reproducible determination of the proliferation activity of CG4 cells. The CCK-8 assay is based on the conversion of tetrazolium salt to formazan by viable cells. The amount of formazan dye generated by mitochondrial enzymes in cells is proportional to the number of living cells. Briefly, after the cultures were treated with various concentrations of QUE, OLA, or HAL (0, 0.01, 0.1, 1.0, 10.0, 100.0 μM) in the growth medium for 24 h, 48 h or 72 h, the viability of CG4 cells was measured by adding 10 μl of the cell counting kit-8 solution into each well of the 96-well microplates, followed by incubation at 37 °C for 4 h. Absorbance was measured at 450 nm using a microplate reader.

Western blot analysis

The CG4 cells were plated on PDL-coated 6-well plates. Cell differentiation was induced by withdrawal of B104-conditioned medium while the cells were being treated with various concentrations of QUE, OLA or HAL for 3 days at 37 °C with 5% CO_2/95% air. Differentiated cells were harvested for Western blot analysis on day 3. Proteins were extracted from differentiated CG4 cells using a RIPA lysis buffer (50 mM Tris–Cl, pH 7.4, 150 mM NaCl, 1% NP40 and 0.25% sodium deoxycholate) with freshly added Protease Inhibitor Cocktail (Sigma-Aldrich, St Louis, MO). Proteins were quantified using a BCA kit (Pierce, Nepean, ON, Canada). Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide (SDS) gel electrophoresis and then transferred to PVDF membranes. The membranes were blocked in 5% skim milk in phosphate-buffered saline (PBS) at 22 °C for 1 h. The membranes were then incubated with the primary antibodies mouse anti-CNP (1:500, Abcam, Cambridge, MA), mouse anti-olig1 (1:2000, Millipore, Billerica, MA) or mouse anti-olig2 (1:2500, Millipore, Billerica, MA). Anti-β-actin antibody (1:5000, Santa Cruz Biotechnology, Santa Cruz, CA) was used as an internal control for the quantity of protein loaded. After washing in PBST (PBS with 0.1% Tween-20), the membranes were incubated with horseradish peroxidase-conjugated goat anti-mouse antibody (1:5000, Invitrogen, Burlington, ON, Canada) at 22 °C for 1 h. The immunoblots were developed using a Western Lightning-ECL kit (PerkinElmer, Waltham, MA). Quantification of the immunoblots was performed using Image-Pro Plus software (version 6.0) and the results were expressed as the ratio of target proteins over β-actin.

Immunocytofluorescent staining and observation

After 3 days of treatment with APDs, CG4 cells on 35 mm PDL-coated Petri dishes were rinsed with PBS and fixed with 4% PFA at 22 °C for 10 min. The fixed cells were then washed with PBS three times, permeabilized and blocked with a blocking buffer containing 0.3% Triton X-100 and 5% normal goat serum in PBS at 22 °C for 30 min. The cells were then incubated with mouse anti-CNP antibody (1:1000, Abcam, Cambridge, MA) in a blocking buffer at 4 °C overnight. Cells were rinsed in PBS 3 times and then incubated with Alexa Fluor®488 goat anti-mouse IgG (H + L) (Invitrogen, Burlington, ON, Canada) at 22 °C for 1 h in the dark. Nuclear DNA was counterstained with Hoechst 33324 (Invitrogen, Burlington, ON, Canada). The coverslips were mounted on the cells with Fluoromount (Sigma, St. Louis, MO) to protect from quenching. The cells were observed under a Nikon TE 2000-E fluorescence microscope (Nikon Instruments Inc., Melville, NY).

Statistical analysis

Three replicates in each cell culture experiment were used for the statistical analysis. Data are expressed as mean ± SEM. Two-way analysis of variance (ANOVA) was performed for the data of CCK-8 assays