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Short communication

Effects of clozapine, olanzapine and haloperidol on nitric oxide production by lipopolysaccharide-activated N9 cells

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Abstract

Schizophrenia is a devastating illness of unknown etiology and the basis for its treatment rests in the symptomatic response to antipsychotics. It was found that some of the patients with schizophrenia elicited microglia activation. The present study used lipopolysaccharide (LPS)-activated mouse microglial cell line N9 as an *in vitro* model to mimic microglia activation seen in the patients with schizophrenia. The effects of clozapine, olanzapine and haloperidol on the release of nitric oxide (NO) by LPS-stimulated N9 cells were investigated. The results showed that olanzapine significantly inhibited NO release by LPS-stimulated N9 cells. Clozapine and haloperidol did not show significant effects on this model. The present study suggested that the inhibiting effect of olanzapine on the NO release by LPS-stimulated microglial cells might be a new mechanism through which olanzapine exhibits its therapeutic effect in the treatment of schizophrenia.

Keywords: Clozapine; Haloperidol; N9 cells; Nitric oxide (NO); Olanzapine

1. Introduction

Schizophrenia is a severe illness that affects approximately 1% of the world's population. The etiology of schizophrenia is only partially understood and, consequently, the basis for its treatment rests in the symptomatic response to antipsychotics. Although classic antipsychotic drugs, such as haloperidol, produce a marked reduction in positive symptoms of schizophrenia, they do not improve the negative symptoms such as apathy, confusion, and social withdrawal, nor do they alter the progressive deterioration in the mental abilities of the patients. In recent years, several new drugs, such as clozapine and olanzapine, have been shown to improve both positive and negative symptoms of schizophrenia, and seem to prevent further worsening of psychotic symptoms (Buckley, 1997; Blin, 1999; Bhana et al., 2001). The atypical antipsychotic drugs are potent 5-HT2A and weak D2 antagonist, which distinguishes them from typical antipsychotic drugs (Altar et al., 1986; Meltzer et al., 1989; Meltzer, 1999; Rasmussen and Aghajanian, 1988). They also have many other pharmacologic properties that may contribute to their superior therapeutic actions in schizophrenia and that could be the basis for their usefulness in controlling psychotic symptoms in other disorders as well (Tran et al., 1997; Stoppe and Staedt, 1999; Wolfgang, 1999).

Microglial cells are ubiquitously distributed in the central nervous system (CNS) and comprise up to 20% of the total glial cell population in the brain (Lawson et al., 1991). As a kind of cells of the macrophage lineage in the CNS, microglial cells are quiescent in the normal brain. However, these cells can be activated by cytokines produced by infiltrating immune effector cells after CNS injury or by LPS during bacterial infection (Gonzalez-Scarano and Baltuch, 1999; Stoll and Jander, 1999). Activation of microglial cells is associated with increased phagocytosis and release of NO, oxygen radicals, proteases as well as pro-inflammatory cytokines (Stoll and Jander, 1999; Lee et al., 2002). In recent years, microglial cells have been shown

Abbreviations: CNS, central nervous system; DMSO, dimethyl sulfoxide; LPS, lipopolysaccharide; NO, nitric oxide; NOS, nitric oxide synthase.

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to be involved in many CNS illnesses. For example, prolonged and excessive stimulation of microglial cells initiates an inflammatory cascade in the CNS that contributes to the pathogenesis of Alzheimer's disease, Parkinson's disease (Stoll and Jander, 1999; Mcgeer et al., 1993), multiple sclerosis (Boyle and McGeer, 1990) and HIV-associated dementia (Kaul et al., 2001). Recently, Munn (2000) has proposed microglia dysfunction in schizophrenia as an integrated theory. The study of Bayer et al. (1999) showed that some of the patients with schizophrenia elicited microglia activation, suggesting microglia activation might be involved in the pathophysiological process of schizophrenia. Taken together, the present study used LPS-activated mouse microglial cell line N9 as an *in vitro* model to mimic microglia activation seen in the patients with schizophrenia. The effects of clozapine, olanzapine and haloperidol on the release of NO by LPS-stimulated N9 cells were investigated.

2. Materials and methods

2.1. Materials

Clozapine and haloperidol were purchased from Sigma (St. Louis, MO, USA). Olanzapine was purified from the tablets (Eli Lilly and Co. Ltd.) by the Department of Pharmaceutical Engineering, Shenyang Pharmaceutical University (Shenyang, China). The drug concentrations were determined according to the results of our preliminary study. In our preliminary study, the highest concentrations tested for haloperidol, olanzapine and clozapine were all up to 100 μM. It was found that haloperidol and clozapine, at the concentrations of 30 and 100 μM, significantly reduced the viability of N9 cells. In contrast, olanzapine (up to 100 μM) did not affect the viability of N9 cells. In order to devoid the possible effects of reduced viability on NO release, the drug concentrations not affecting the viability of N9 cells were selected in the present study. Fetal bovine serum (FBS) was purchased from TBD Biotechnology Development (Tianjin, China). LPS (E5:055) was purchased from Sigma (St. Louis, MO, USA). Thiazolylblue (MTT) was from Sino-American Biotechnology (Beijing, China). Iscove's modified dulbecco's medium (IMDM) was from Gibco (Grand Island, USA). Clozapine, olanzapine and haloperidol were dissolved initially in dimethyl sulfoxide (DMSO) and were diluted with PBS for experiments. DMSO at the highest concentration possibly present in experimental conditions (0.1%) was not toxic to cells.

2.2. Cell culture

The murine microglial cell line N9 was a kind gift from Dr. J. M. Wang (NCI, Frederick, USA). The cells were grown in IMDM supplemented with 5% heat-inactivated FBS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5×10^{-5} M 2-mercaptoethanol.

Cells at density of 3×10^{4} cells/well were plated onto 96-well microtiter plates for MTT and nitrite assay. Clozapine, olanzapine or haloperidol with or without LPS (1 μg/ml) was added to the culture medium of N9 cells for 24 h for the experiments. The drug doses used were according to our preliminary study, not affecting the viability of N9 cells.

2.3. Cell viability

Cell viability was evaluated by the MTT reduction assay (Chang et al., 1998). In brief, cells were seeded onto 96-well microtiter plates and treated with various reagents for the indicated time period. After various treatments, medium was removed and the cells were incubated with MTT (0.25 mg/ml) for 3 h at 37 °C. The formazan crystals in the cells were solubilized with a solution containing 50% dimethylformamide and 20% sodium dodecyl sulfate (pH 4.7). The level of MTT formazan was determined by measuring its absorbance at the wavelength of 490 nm with a SPECTRA (shell) Reader (TECAN, Austria).

2.4. Nitrite assays

Accumulation of nitrite (NO_2^-) in the culture supernatants, an indicator of NO synthase activity, was measured by Griess reaction (Barger and Harmon, 1997). Fifty microliter culture supernatants were mixed with 50 μl Griess reagent (part I: 1% sulfanilamide; part II: 0.1% naphthylethylene diamide dihydrochloride and 2% phosphoric acid) at room temperature. Fifteen minutes later, the absorbance was determined at 540 nm using the SPECTRA (shell) Reader. Nitrite concentration was calculated with reference to a standard curve of sodium nitrite generated by known concentrations.

2.5. Data analysis

Results were expressed as mean±S.E.M. Statistical significance ($P<0.05$) was assessed by one-way ANOVA followed by Dunnett's *t*-test (SPSS12.0 software, SPSS, USA).

3. Results

3.1. The effect of clozapine on N9 cell viability and LPS-induced NO release

Treatment with clozapine (1–10 μM) alone or with 1 μg/ml of LPS for 24 h did not cause any change in MTT absorbance in N9 cells, indicating that clozapine did not affect the viability of N9 cells at the doses used (Fig. 1).

Next, the release of NO by LPS-stimulated N9 cells was examined. In unstimulated N9 cells, only small amounts of NO_2^- (4.23±2.18 μM) occurred in the medium. Pretreatment of unstimulated cells with clozapine (1–10 μM) for 24 h did not result in any change of NO_2^-. The stimulation of N9 cells with LPS resulted in a manifold increase in NO_2^- production (11.1± 5.1 μM). LPS significantly induced NO release in N9 cells, which was in consistence with the previous report (Bi et al., 2005). Simultaneous treatment of N9 cells with LPS and clozapine (1–10 μM) was not different from the result observed by LPS alone.