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Ziprasidone attenuates brain injury after focal cerebral ischemia induced by middle cerebral artery occlusion in rats

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ABSTRACT

Ziprasidone is an atypical antipsychotic drug used for the treatment of schizophrenia. Recent studies have reported that atypical antipsychotics have neuroprotective effects against brain injury. In the present study, the effect of ziprasidone on ischemic brain injury was investigated. Focal cerebral ischemia was induced by middle cerebral artery occlusion (MCAO) in rats. All the animals experienced ischemia for 1 h and then underwent reperfusion. The infarct size induced by MCAO was significantly reduced in the animals that received acute treatment with 5 mg/kg ziprasidone and subchronic treatment with 2.5 mg/kg ziprasidone for 7 days compared with that in the vehicle-treated animals. The acute treatment with ziprasidone significantly improved neurological functions, as measured by the modified neurological severity score, in a dose-dependent manner. The subchronic treatment produced more rapid recovery from functional deficits than the vehicle treatment. The immunohistochemical investigation revealed that the subchronic treatment prevented severe loss of neuronal marker intensity and attenuated the increased in microglial marker intensity in the infarcted cortical area. These results suggest that ziprasidone has neuroprotective effects in a rat model of ischemic stroke and provide new insight for its clinical applications.

1. Introduction

Cerebral ischemia is one of the leading causes of death and disability in adults worldwide. The primary consequences of this event are significant reductions in blood flow and nutrients critical for neural function and survival. Preclinical and clinical studies report that memory impairment, cognitive deficits, and brain damage are observed in patients with cerebral ischemia (Kruyt et al., 2008; Nunn and Hodges, 1994). Hence, it is believed that ischemia-induced brain damage is associated with cognitive and memory dysfunction (Li et al., 2009; Oksala et al., 2009). In addition to gaining insight into the endogenous neuroprotective capacity of the brain, recent basic neuroscience research has focused on developing clinically and experimentally applicable neuroprotective drugs for stroke, including atypical antipsychotic drugs, such as olanzapine (Yulug et al., 2006) and quetiapine (Yan et al., 2007).

Ziprasidone is a member of the benzisothiazolyl piperazine family that was developed from the chemically related antipsychotic drug, tiospirone (Seeger et al., 1995). This atypical antipsychotic agent has been approved by the Food and Drug Administration (FDA) for the acute treatment of schizophrenia and schizoaffective disorders; it has minimal adverse effects on motor, cognitive, prolactin-related, and anticholinergic functions, as well as weight (Daniel and Copeland, 2000; Nemeroff et al., 2005). Ziprasidone was subsequently approved by the FDA for the treatment of acute bipolar mania (Nemeroff et al., 2005; Sachs et al., 2011). There is also some evidence that ziprasidone may be efficacious in the treatment of agitation and/or aggression in patients with dementia (Cole et al., 2005) or traumatic brain injury (Scott et al., 2009), as well as in the treatment of delirium (Young and Lujan, 2004). Ziprasidone has a unique pharmacological profile, as it has antagonist activity at dopamine (DA) D_2 and serotonin (5-HT) 5-HT_{1D}, 5-HT_{2A}, and 5-HT_{2C} receptors and partial agonist activity at 5-HT_{1A} receptors (Nemeroff et al., 2005). It is also a relatively potent inhibitor of 5-HT and norepinephrine reuptake in vitro, making it a unique atypical antipsychotic drug (Schmidt et al., 2001).

Abbreviations: 5-HT, serotonin; DA, dopamine; FDA, Food and Drug Administration; MCAO, middle cerebral artery occlusion; mNSS, modified neurological severity score; TTC, 2,3,5-triphenyltetrazolium chloride.

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Increasing evidence indicates that atypical antipsychotic agents may have neuroprotective effects in addition to their common effect of reducing schizophrenic symptoms. Recently, several studies demonstrated that these drugs can improve brain impairment after damage. Olanzapine decreases the infarct volume after focal cerebral ischemia (Yulug et al., 2006). Quetiapine attenuates cognitive and non-cognitive behavioral impairment induced by global cerebral ischemia (He et al., 2009) and reverses the suppression of hippocampal neurogenesis caused by repeated restraint stress (Luo et al., 2005). Several mechanisms explaining the neuroprotective actions of atypical antipsychotics have been examined. Chronic administration of clozapine and olanzapine upregulates the levels of brain-derived neurotrophic factor in the rat brain (Bai et al., 2003). These drugs also upregulate bcl-2 mRNA and protein in rat frontal cortex and hippocampus (Bai et al., 2004). As schizophrenia is associated with several immunological abnormalities, it has also been demonstrated that typical and atypical antipsychotics modulate the production of cytokines (Sugino et al., 2009). However, the effects of ziprasidone on brain damage have rarely been investigated.

We investigated the effects of ziprasidone administered after the induction of ischemic injury in a rat model of cerebral ischemia. We used middle cerebral artery occlusion (MCAO) to induce transient ischemia in rats. Neuroprotection was evaluated according to infarct volume measurements, neurological functions, and immunohistochemical features.

2. Methods

2.1. Animals

All the animals were manipulated in accordance with the animal care guidelines of the US National Institutes of Health and the Korean Academy of Medical Science. All the experimental procedures were approved by the Committee for Animal Experimentation and the Institutional Animal Laboratory Review Board of Inje University. Adult (9-week-old) male Sprague–Dawley rats weighing 280–320 g at the time of surgery were used in this study. The rats were housed 3 per cage in a room maintained at 21 °C, with a 12/12-h light–dark cycle, and had free access to food and water. After 7 days of acclimatization, the rats were subjected to cerebral ischemia by MCAO. The rats were assigned to 2 experiments: experiment 1 investigated the acute (24-h) effect of ziprasidone, and experiment 2 investigated the subchronic (7-day) effects of ziprasidone.

2.2. Drug and treatment

Ziprasidone was dissolved in distilled water with 0.4% glacial acetic acid. The rats in experiment 1 were divided into 3 groups: group 1 received 0.4% glacial acetic acid in distilled water as a vehicle (1 mL/kg, intraperitoneally [i.p.]), whereas groups 2 and 3 received 2.5- and 5.0-mg/kg ziprasidone i.p., respectively, in 1-mL/kg vehicle. The treatments in all the groups were started 1 h after MCAO, followed by reperfusion. The animals were killed 24 h after the treatment.

In experiment 2, the animals were first given ziprasidone (2.5 mg/kg, 1 mL/kg, i.p.) or the vehicle 1 h after MCAO, followed by reperfusion; thereafter, drug administration was performed daily for 7 days. The animals were killed 24 h after the last injection. The doses of the drug to be administered were selected on the basis of previous animal studies (Abdul-Monim et al., 2006) and were concordant with information in published reports regarding the occupancy of dopamine receptors (Barth et al., 2006; Park et al., 2009). Ziprasidone was generously supplied by Pfizer Pharmaceuticals (New York, NY, USA).

2.3. Induction of focal cerebral ischemia

Focal cerebral ischemia was induced via MCAO, which was performed as described previously with some modifications (Longa et al., 1989; Nagasawa and Kogure, 1989). Briefly, the animals were anesthetized with a mixture of ketamine (1.5 mL/kg) and xylazine (0.5 mL/kg). The right common carotid artery was exposed and carefully dissected free of the vagus nerve. The right external and internal carotid arteries were also isolated. The external carotid artery was then ligated at the distal end, which was then cut off. A 4-0 nylon thread precoated with silicon resin (Xantopren Bayer Dental, Osaka, Japan) was aseptically introduced into the right carotid artery in an anterograde fashion toward the carotid bifurcation; it was then directed distally up to the right internal carotid artery to a point approximately 20 mm from the carotid bifurcation to occlude the origin of the middle cerebral artery. After 1 h, the thread was withdrawn for reperfusion. All the animals experienced ischemia for 1 h followed by reperfusion. Rectal temperature was maintained at 37 ± 0.5 °C throughout the surgical procedure by using a thermostatically controlled warming plate and overhead lamp.

2.4. Cerebral infarction measurement

Each group of animals in experiments 1 and 2 was decapitated 24 h after the last drug treatment. The brain was carefully removed, dissected into coronal sections 2 mm thick in a metallic brain matrix, immersed sequentially in a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) in normal saline at 37 °C for 10 min, and fixed in 10% formalin. The infarct area in the brain section 1 mm posterior to the bregma was measured using an image analyzer (UTHSCSA ImageTool, San Antonio, TX, USA).

2.5. Behavioral functional test

For the animals in experiment 1, behavioral tests were performed 24 h after ziprasidone administration. Meanwhile, in experiment 2, the behavioral tests were conducted on days 0, 1, 3, and 7 after MCAO. The behavioral test on day 0 was conducted 1 h before MCAO, when the animals were exhibiting no neurological deficits. The behavioral tests were conducted by an investigator blinded to which experimental group each animal belonged. All rats were evaluated using the modified neurological severity score (mNSS) (Chen et al., 2001). The mNSS is a composite of motor (muscle status and abnormal movement), sensory (visual, tactile, and proprioceptive), reflex, and balance tests. Neurological function was graded on a scale of 0 to 18 (normal score, 0; maximal deficit score, 18). For the injury severity scores, 1 score point was awarded for the inability to perform a test or for the lack of a tested reflex. Thus, higher scores indicate more severe injuries.

2.6. Immunohistochemistry

The animals given ziprasidone (2.5 mg/kg, 1 mL/kg, i.p.) daily for 7 days were killed 24 h after the last injection and then fixed by cardiac perfusion with 4% paraformaldehyde in 0.1-M phosphate buffer. The brains were then sliced into coronal sections (10 μm thick) by a cryostat (HM 525, Thermo Scientific, MA). After blocking with goat serum for 2 h, the sections were incubated with primary antibodies against NeuN (1:700, Chemicon, CA) and Iba-1 (1:500, Wako, Japan) diluted in Tris-buffered saline (TBS) containing 1% bovine serum albumin (*w*/*v*) and 0.3% Triton-X 100. The sections were incubated overnight at 4 °C. After rinsing in TBS, the sections were incubated with secondary antibody-conjugated FITC (1:200, Sigma-Aldrich) and Cy3 (1:200, Amersham, USA) for NeuN and Iba-1, respectively, for 2 h at room temperature. The sections were then rinsed in TBS and mounted in an aqueous mounting medium.

2.7. Statistical analysis

The infarct area data and neurological deficit scores are expressed as mean ± SE. Student *t* test was used to compare the differences in