Effects of Memantine on Nitric Oxide Production and Hydroxyl Radical Metabolism during Cerebral Ischemia and Reperfusion in Mice

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Background: The purpose of this study was to investigate the effects of memantine on brain ischemia. Because we can measure nitric oxide (NO) production and hydroxyl radical metabolism continuously, we investigated the effect of memantine on NO production and hydroxyl radical metabolism in cerebral ischemia and reperfusion. *Methods:* Memantine (25 μmol/kg) was administered intraperitoneally to 6 C57BL/6 mice 30 minutes before ischemia. Seven additional mice received no injection (controls). NO production and hydroxyl radical metabolism were continuously monitored using bilateral striatal microdialysis in vivo. Hydroxyl radical formation was monitored using the salicylate trapping method. Forebrain ischemia was produced in all mice by occluding the common carotid artery bilaterally for 10 minutes. Levels of the NO metabolites nitrite (NO_2^-) and nitrate (NO_3^-) were determined using the Griess reaction. Survival rates of hippocampal CA1 neurons were calculated and 8-hydroxydeoxyguanosine (8-OHdG)-immunopositive cells were counted to evaluate the oxidative stress in hippocampal CA1 neurons 72 hours after the start of reperfusion. *Results:* The regional cerebral blood flow was significantly higher in the memantine group than in the control group after reperfusion. Furthermore, the level of 2,3-dihydroxybenzoic acid was significantly lower in the memantine group than in the control group during ischemia and reperfusion. Levels of NO_2^- and NO_3^- did not differ significantly between the 2 groups. Although survival rates in the CA1 did not differ significantly, there were fewer 8-OHdG-immunopositive cells in animals that had received memantine than in control animals. *Conclusions:* These data suggest that memantine exerts partially neuroprotective effects against cerebral ischemic injury. **Key Words:** Nitric oxide (NO)—hydroxyl radical—memantine—microdialysis—global ischemia—CA1 neuron.

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Introduction

The glutamate-mediated neurotoxicity hypothesis of the pathomechanism of Alzheimer's disease (AD) is widely accepted. The N-methyl-D-aspartate receptor (NMDA-R) antagonist memantine, used in the treatment of AD, has a neuroprotective effect, blocking NMDA-induced excitotoxicity[1] and reducing the symptoms of dementia.

The neuroprotective effects of memantine have been clinically demonstrated in the field of dementia. Memantine protects neurons against death from transient frontal brain ischemia[2] and infantile rat hydrocephalus, especially in the CA1 and CA2 areas of the hippocampus.[3] In addition, memantine significantly reduces infarct size after hypoxia-ischemia in rats.[4] Memantine has neuroprotective effects in a photothrombotic model of cerebral focal ischemia in rats[5] and prevents neuronal death after global cerebral ischemia and reperfusion in mice.[6] Furthermore, memantine decreases hippocampal neuronal cell death after traumatic brain injury in rats.[7]

In ischemia, a large amount of glutamate is released, causing excessive stimulation of NMDA-R and increased interaction between neuronal NOS and postsynaptic density protein 95.[8,9] During cerebral ischemia, the NMDA-R forms a death-signaling complex with postsynaptic density protein 95 and neuronal NOS, leading to calcium-dependent production of the superoxide anion (O_2^-) and NO. The formation of peroxynitrite ($ONOO^-$) from O_2^- and NO can induce neuronal injury via several mechanisms.[9,10]

However, the effects of memantine on nitric oxide (NO) production and hydroxyl radical metabolism during cerebral ischemia and reperfusion in vivo have never been investigated. The aim of the present study was to investigate the effects of memantine on NO production, hydroxyl radical metabolism, and ischemic changes in hippocampal CA1 neurons during cerebral ischemia and reperfusion in mice using in vivo microdialysis.

Experimental Procedures

Materials

All reagents were purchased from Sigma-Aldrich (Tokyo, Japan), unless otherwise stated.

Animal Preparation

C57BL/6 mice (n = 13) (Charles-River; Atsugi, Kanagawa, Japan) were housed in the animal care facility at Saitama Medical University. Animals were initially anesthetized with 2% halothane in air supplemented with O_2 and maintained with .5%-1% halothane. Rectal temperature was maintained at 37.0°C-37.5°C with a disposable heat pack and small fan. A polyethylene catheter (PE-10, BD, Japan) was inserted into the right femoral artery to measure blood pressure.

Memantine (25 μmol/kg) was dissolved in physiological saline and administered intraperitoneally to six mice 30 minutes before ischemia. The remaining seven mice served as controls without memantine. All animal experiments were approved by the Institutional Animal Care and Use Committee of Saitama Medical University, Japan (approval numbers: 1658 in 2015 and 1929 in 2016).

In Vivo Microdialysis

NO production and hydroxyl radical metabolism were monitored continuously using in vivo microdialysis. Mice in both groups were initially anesthetized with 2% halothane in air supplemented with O_2, then maintained with .5%-1% halothane[8] (Fig 1, A). A microdialysis probe was inserted into the striatum in each hemisphere and perfused with Ringer's solution at a constant rate of 2 μL/min. The in vivo salicylate trapping method was used to monitor hydroxyl radical formation via 2,3-dihydroxybenzoic acid (2,3-DHBA) and 2,5-dihydroxybenzoic acid (2,5-DHBA) (Fig 1, B). A laser Doppler probe was placed on the surface of the skull of the right hemisphere. After 2 hours of equilibration, fractions were collected every 10 minutes (Fig 1, A). Global forebrain cerebral ischemia was produced by occlusion of both common carotid arteries using Micro Head clips for 10 minutes.

All dialysis equipment was from Eicom, Kyoto, Japan, unless otherwise stated. Levels of nitrite (NO_2^-) and nitrate (NO_3^-) in the dialysates were determined using an ENO-20 high performance liquid chromatography system, M-510 automatic sample injector. Samples were analyzed using the Griess reaction, in which NO_2^- and NO_3^- were separated on a packed column (Eicom, kyoto, Japan, NO-PAK), and NO_3^- was reduced to NO_2^- in a cadmium reduction column (Eicom, kyoto, Japan, NO-RED).

Dialysates were collected into an autoinjector (Eicom, kyoto, Japan, EAS-20) every 20 minutes, and 2,3- and 2,5-DHBA levels were measured using an high performance liquid chromatography system equipped with an electrochemical detector (Eicom, kyoto, Japan, HITEC-500) consisting of a graphite working electrode at +500 mV versus an Ag/AgCl reference electrode. Separation was done on an Eicompac SC-5ODS (Eicom, kyoto, Japan) column (2.1 mm × 150 mm) at 25°C with a mobile phase consisting of 100 mM sodium phosphate buffer (pH 6.0) containing 134 μM EDTA and 2% methanol, at a flow rate of 230 μL/min. The locations of the dialysis probes were verified after each experiment.

Salicylic acid reacts with hydroxyl radicals to form 2 main adducts, 2,3-DHBA and 2,5-DHBA.[11] Because 2,5-DHBA is also endogenously formed by the cytochrome *P*450 system, only the changes in 2,3-DHBA are a reliable hydroxyl marker in vivo.[12]

Morphological Classification of Neurons

At 72 hours after the start of reperfusion, mice in the memantine and the control groups were transcardially perfused with .9% saline followed by 4% paraformaldehyde.