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Pre- and post-treatments with escitalopram protect against experimental ischemic neuronal damage via regulation of BDNF expression and oxidative stress

Choong Hyun Lee [a,1], Joon Ha Park [b,1], Ki-Yeon Yoo [c], Jung Hoon Choi [d], In Koo Hwang [e,f], Pan Dong Ryu [a], Do-Hoon Kim [g], Young-Guen Kwon [h], Young-Myeong Kim [i,j], Moo-Ho Won [b,*]

[a] Laboratory of Veterinary Pharmacology, College of Veterinary Medicine, Seoul National University, Seoul 151–742, South Korea
[b] Department of Neurobiology, School of Medicine, Kangwon National University, Chuncheon 200–701, South Korea
[c] Department of Oral Anatomy, College of Dentistry, Gangneung-Wonju National University, Gangneung 210–702, South Korea
[d] Department of Anatomy, College of Veterinary Medicine, Kangwon National University, Chuncheon 200–701, South Korea
[e] Department of Anatomy and Cell Biology, College of Veterinary Medicine, Seoul National University, Seoul 151–742, South Korea
[f] Research Institute for Veterinary Science, Seoul National University, Seoul 151–742, South Korea
[g] Department of Psychiatry, Chuncheon Sacred Heart Hospital, College of Medicine, Hallym University, Chuncheon 200–704, South Korea
[h] Department of Biochemistry, College of Sciences, Yonsei University, Seoul 120–749, South Korea
[i] Vascular System Research Center, School of Medicine, Kangwon National University, Chuncheon 200–701, South Korea
[j] Department of Molecular and Cellular Biochemistry, School of Medicine, Kangwon National University, Chuncheon 200–701, South Korea

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ABSTRACT

Selective serotonin re-uptake inhibitors (SSRI) have been widely used in treatment of major depression because of their efficacy, safety, and tolerability. Escitalopram, an SSRI, is known to decrease oxidative stress in chronic stress animal models. In the present study, we examined the neuroprotective effects of pre- and post-treatments with 20 mg/kg and 30 mg/kg escitalopram in the gerbil hippocampal CA1 region (CA1) after transient cerebral ischemia. Pre-treatment with escitalopram protected against ischemia-induced neuronal death in the CA1 after ischemia/reperfusion (I/R). Post-treatment with 30 mg/kg, not 20 mg/kg, escitalopram had a neuroprotective effect against ischemic damage. In addition, 20 mg/kg pre- and 30 mg/kg post-treatments with escitalopram increased brain-derived neurotrophic factor (BDNF) protein levels in the ischemic CA1 compared to vehicle-treated ischemia animals. In addition, 20 mg/kg pre- and 30 mg/kg post-treatments with escitalopram reduced microglia activation and decreased 4-hydroxy-2-nonenal and Cu,Zn-superoxide dismutase immunoreactivity and their levels in the ischemic CA1 compared to vehicle-treated ischemia animals after transient cerebral ischemia. In conclusion, these results indicated that pre- and post-treatments with escitalopram can protect against ischemia-induced neuronal death in the CA1 induced by transient cerebral ischemic damage by increase of BDNF as well as decrease of microglia activation and oxidative stress.

Introduction

Transient cerebral ischemia results in the insidious delayed degeneration of specific vulnerable neurons in some brain regions, such as the hippocampus, neocortex, and striatum (Globus et al., 1991; Kirino, 1982; Lin et al., 1990). Among them, the hippocampal CA1 region (CA1) is known as the most vulnerable brain region (Globus et al., 1991). Neuronal death in the CA1 occurs some days after the initial ischemic insult and is referred to as "delayed neuronal death" (Kirino, 1982). However, the underlying mechanisms related to the delayed neuronal death has not been elucidated exactly yet. One of the acceptable mechanisms in ischemia-induced neuronal damage is oxidative stress with reactive oxygen species (ROS) generation like in other brain injuries (Candelario-Jalil et al., 2003; Chan, 2004; Nazıroğlu, 2009; Rastogi et al., 2006). ROS, generated by mitochondrial dysfunctions and overproduced after ischemia/reperfusion (I/R), has been known to play an important role in the process of cell death after ischemic damage (Chan, 2004; McCord, 1985; Moro et al., 2005).

Selective serotonin re-uptake inhibitors (SSRI), such as fluoxetine, citalopram, and escitalopram, have been widely used in the treatment of major depression because of their efficacy, safety, and tolerability (Anderson, 1998; Edwards and Anderson, 1999). SSRI are also used to prevent the onset of post-stroke mood disorder (Paolucci et al., 2001; Robinson et al., 2008; Wiart et al., 2000). It has been known that SSRI have effects on neuronal plasticity in the brain via the increase of brain-derived neurotrophic factor (BDNF) expression (Maya Vetencourt et al., 2008). Moreover, the neuroprotective effects of SSRI, such as fluoxetine and citalopram, have been well established in brain damage induced by various insults (Chang et al., 2006; Jin et al., 2009; Kim do et al., 2007;

* Corresponding author. Fax: +82 33 256 1614.
E-mail address: mhwon@kangwon.ac.kr (M.-H. Won).
[1] Choong Hyun Lee and Joon Ha Park contributed equally to this article.

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Lim et al., 2009; Nakata et al., 1992; Nakata et al., 1997; Sas et al., 2007). Among SSRI, escitalopram, S-enantiomer of citalopram, shows a faster time to onset of efficacy than citalopram (Montgomery et al., 2001). Escitalopram is also known to decrease oxidative stress in chronic stress models of mice and rats (Eren et al., 2007; Kumar and Garg, 2009).

Although many investigators have focused on the protective effects of SSRI against brain damage including ischemia, there are small numbers of studies on the neuroprotective effect of citalopram, not escitalopram, following cerebral ischemia (Nakata et al., 1992, 1997). In addition, there is no study on whether post-treatment with escitalopram has a neuroprotective effect on ischemic neuronal damage. In the present study, therefore, we examined the neuroprotective effects of pre- and post-treatments with escitalopram in the gerbil CA1 after transient cerebral ischemia. We also investigated whether the neuroprotective effects of escitalopram might be associated with changes in BDNF expression and oxidative stress.

Materials and methods

Experimental animals

Male Mongolian gerbils (*Meriones unguiculatus*) were obtained from the Experimental Animal Center, Hallym University, Chuncheon, South Korea. Gerbils were used at 6 months (B.W., 65–75 g) of age. The animals were housed in a conventional state under adequate temperature (23 °C) and humidity (60%) control with a 12-h light/12-h dark cycle, and provided with free access to water and food. All experimental procedures were in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

Treatment with escitalopram

To elucidate the neuroprotective effects of escitalopram (Lexapro, Lundbeck, Denmark) against ischemic damage, the animals were divided into 10 groups ($n = 12$ in each group), as follows: 1) vehicle-treated, sham-operated group (vehicle-sham-group); 2) vehicle-treated, ischemia-operated group (vehicle-ischemia-group); 3) 20 and 30 mg/kg escitalopram-pretreated, sham-operated group (pre-escitalopram-sham-group); 4) 20 and 30 mg/kg escitalopram-pre-treated, ischemia-operated group (pre-escitalopram-ischemia-group); 5) 20 and 30 mg/kg escitalopram-post-treated sham-operated group (post-escitalopram-sham-group); and 6) 20 and 30 mg/kg escitalopram-post-treated ischemia-operated group (post-escitalopram-ischemia-group). Doses of escitalopram were selected based on the previous studies (Nagao et al., 1995; Nakata et al., 1992). Escitalopram was dissolved in saline. For pretreatment, escitalopram or saline was administered intraperitoneally once daily for 3 days before ischemic surgery: last treatment was at 30 min before surgery. For post-treatment, escitalopram or saline was administered intraperitoneally at 30 min and 1 day after reperfusion. For the experiment of changes in 4-hydroxy-2-nonenal (HNE) and Cu,Zn-superoxide dismutase (SOD)1, escitalopram or saline was administered only at 30 min after reperfusion in the post-treated groups.

Induction of transient cerebral ischemia

The animals were anesthetized with a mixture of 2.5% isoflurane (Baxtor, Deerfield, IL) in 33% oxygen and 67% nitrous oxide. Bilateral common carotid arteries were isolated and occluded using non-traumatic aneurysm clips. The complete interruption of blood flow was confirmed by observing the central artery in retinae using an ophthalmoscope. After 5 min of occlusion, the aneurysm clips were removed from the common carotid arteries. The body (rectal) temperature under free-regulating or normothermic (37 ± 0.5 °C) conditions was monitored with a rectal temperature probe (TR-100; Fine Science Tools, Foster City, CA) and maintained using a thermometric blanket before, during, and after the surgery until the animals completely recovered from anesthesia. Thereafter, animals were kept on the thermal incubator (Mirae Medical Industry, Seoul, South Korea) to maintain the body temperature of animals until the animals were euthanized. Sham-operated animals were subjected to the same surgical procedures except that the common carotid arteries were not occluded.

Tissue processing for histology

For the histological analysis, animals were anesthetized with sodium pentobarbital and perfused transcardially with 0.1 M phosphate-buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate-buffer (PB; pH 7.4). The brains were removed and postfixed in the same fixative for 6 h. The brain tissues were cryoprotected by infiltration with 30% sucrose overnight. Thereafter, frozen tissues were serially sectioned on a cryostat (Leica, Wetzlar, Germany) into 30-μm coronal sections, and they were then collected into 6-well plates containing PBS.

Staining for NeuN and Fluoro-Jade B

To examine neuronal damage in the hippocampal CA1 region after I/R, animals were used at 4 days after I/R. NeuN (a marker for neuron) immunohistochemistry and Fluoro-Jade B (F-J B, a useful marker for neuronal degeneration) histofluorescence staining were done according to the method of the previous study (Kim do et al., 2007; Schmued and Hopkins, 2000). In brief, the sections were incubated with diluted mouse anti-NeuN (1:1000; Chemicon, Temecula, CA) and subsequently exposed to biotinylated goat anti-mouse IgG and streptavidin peroxidase complex (1:200; Vector, Burlingame, CA). Moreover, they were visualized by staining with 3,3′-diaminobenzidine (Sigma) in 0.1 M Tris–HCl buffer (pH 7.2). For F-J B staining, the sections were first immersed in a solution containing 1% sodium hydroxide in 80% alcohol and followed in 70% alcohol. They were then transferred to a solution of 0.06% potassium permanganate and transferred to a 0.0004% Fluoro-Jade B (Histochem, Jefferson, AR) staining solution. After washing, the sections were placed on a slide warmer (approximately 50 °C) and then examined using an epifluorescent microscope (Carl Zeiss, Göttingen, Germany) with blue (450–490 nm) excitation light and a barrier filter.

To evaluate the neuroprotective effect of escitalopram, NeuN-immunoreactive neurons were counted in a 250×250 μm square applied approximately at the center of the CA1 using an image analyzing system (software: Optimas 6.5; CyberMetrics, Scottsdale, AZ). The studied tissue sections were selected with 180-μm intervals, and cell counts were obtained by averaging the counts from each animal.

Immunohistochemistry for Iba-1, HNE, and SOD1

Immunohistochemical staining for rabbit anti-ionized calcium-binding adapter molecule 1 (Iba-1, 1:200; Wako, Osaka, Japan) for microglia, mouse anti-HNE (1:1000; Alexis Biochemicals, San Diego, CA) for lipid peroxidation, and mouse-anti-SOD1 (1:1000; Calbiochem, Germany) for antioxidant enzyme were performed. A negative control test was carried out using pre-immune serum instead of primary antibody in order to establish the specificity of the immunostaining. The negative control resulted in the absence of immunoreactivity in all structures.

In order to quantitatively analyze the immunoreactive structures, semi-quantification of the immunostaining intensities was evaluated with digital image analysis software (MetaMorph 4.01; Universal Imaging Corp.). The mean intensity of immunostaining in each immunoreactive structures was measured by a 0–255 gray scale