Estrogen Modulates Bcl-2 Family Proteins in Ischemic Brain Injury

Chung-Kil WON1), Myeong-Ok KIM2) and Phil-Ok KOH1)*

1) Department of Anatomy, College of Veterinary Medicine and Institute of Animal Medicine, Gyeongsang National University, 900 Gajwa-dong, Jinju 660-701 and 2) Division of Life Science, College of Natural Sciences, Gyeongsang National University, Jinju 660-701, South Korea

Received 5 August 2005/Accepted 24 October 2005

ABSTRACT. Estradiol acts as a neuroprotective factor against brain injury. This study investigated whether estradiol modulates the Bcl-2 family proteins in ischemic brain injury. Adult female rats were ovariectomized and treated with estradiol prior to middle cerebral artery occlusion (MCAO). Brains were collected 24 hr after MCAO, and infarct volumes were analyzed. Estradiol significantly reduces the infarct volume and decreases the positive cells of TUNEL staining in cerebral cortex. In ischemic cerebral cortex, the level of Bcl-2 was decreased, and the level of Bax was significantly increased. Estradiol prevents the injury-induced decrease of Bcl-2 and increase of Bax. In conclusion, our findings suggest that estradiol plays a potent protective role in brain injury through the regulation of Bcl-2 family proteins.

KEY WORDS: Bax, Bcl-2, Estradiol.

*CORRESPONDENCE TO: KOH, P.O., Department of Anatomy, College of Veterinary Medicine, Gyeongsang National University, Jinju 660-701, South Korea.

Estradiol plays potent neurotropic and neuroprotective roles in immature and adult brains [9, 19]. In particular, clinical studies have shown that estradiol decreased the risk or severity of neurodegenerative conditions such as Alzheimer’s disease [1], stroke [11], and Parkinson’s disease [14]. Estradiol prevents cell death as a response to a variety of neuronal stimuli, including excitotoxic amino acids, β-amyloid toxicity, and oxidative stress [3, 5]. Also, estradiol inhibits cell death caused by ischemic brain injury, by decreasing the extent of apoptotic cell death [4].

The ischemic brain damage induced neuronal cell death through the activation of apoptotic signal pathways, including Bcl-2 family and caspase [7]. It is accepted that Bcl-2 is an anti-apoptotic factor that inhibits apoptotic cell death, whereas Bax is a pro-apoptotic factor that accelerates apoptotic death [2]. In neuronal cells, the overexpression of Bcl-2 protects the neuronal cell death against both transient and permanent ischemic injury [8]. Moreover, ischemic brain injury induced the activation of Bax and other member of the Bcl-2 family [13]. This study examined the neuroprotective effect of estradiol against stroke-like ischemic injury. Further, we investigated whether estradiol modulates the Bcl-2 family proteins, including Bcl-2, Bax and Bel-x(L).

Female Sprague-Dawley rats (225-250 g, 12 weeks age, n=60) were purchased from Samtako Co (Animal Breeding Center, Korea) and were randomly divided into two groups, oil-treated group and estradiol-treated group. Animals were maintained under controlled temperature (25°C) and lighting (14/10 light/dark cycle), and were allowed to have free access to food and water. To eliminate endogenous estradiol production, rats were bilaterally ovariectomized using a dorsal approach. And then implanted with a silastic capsule containing sesame oil (Sigma, St. Louis, MO, U.S.A.) or 17β-estradiol (180 µg/ml, Sigma). This paradigm of estradiol treatment produces levels of estradiol equivalent to basal circulating levels observed during the rat estrous cycles [3]. Two weeks after ovariectomy and treatment, rats underwent middle cerebral artery occlusion (MCAO) to induce cerebral ischemia.

Before surgery, animals were anesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg). MCAO was carried out as described previously [6]. Briefly, the right common carotid artery, external carotid artery, and internal carotid artery were exposed through a midline cervical incision. A 4/0 monofilament nylon suture with its tip slightly rounded by heat was inserted through the internal carotid artery to the base of the middle cerebral artery, thus occluding blood flow to the cortex and striatum. At 24 hr after the onset of permanent occlusion, animals were decapitated, and the brains were removed. These brains were cut into 2 mm thick coronal slices at the optic chiasm level, the coronal levels at which the largest ischemic infarct is observed [15].

These slices were incubated for 20 min in a 2% triphenyltetrazolium chloride (TTC; Sigma) and fixed in 10% formalin. The stained slices were photographed by a Nikon CoolPIX990 digital camera and measured for the ischemic lesion by Image-ProPlus 4.0 software (Media Cybernetics, Silver Spring, MD, U.S.A.). The largest ischemic lesion in brain region was measured, respectively (n=15 per group). The ischemic lesion percentage of each slice was calculated by the ratio of the infarction area to the whole slice area. After TTC staining and lesion area analysis, the slices were embedded with paraffin and sectioned for TUNEL staining. TUNEL histochemistry was performed using the DNA Fragmentation Detection Kit (Oncogene Research Products, Cambrige, MA, U.S.A.). In the TUNEL stained sections, one field for each section was selected from cortex and striatum. TUNEL-positive cells were quantified using light
For western blot analysis, the slices were dissected into ipsilateral and contralateral cerebral cortices (n=15 per group). Samples were snap frozen and lysed in buffer. The protein concentration of each lysate was determined using the bicinchoninic acid (BCA) kit (Pierce, Rockford, IL, U.S.A.) according to the manufacturer’s protocol. Total protein (30 μg) was applied to each lane on 10% SDS-polyacrylamide gels. After electrophoresis, the polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, U.S.A.) were washed in Tris-buffered saline containing 0.1% Tween-20 and then incubated with anti-Bcl-2, Bax, and Bcl-x(L) antibody (diluted 1:1,000, Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) as primary antibody. And the membrane was incubated with secondary antibody (1:5,000, Pierce) and the ECL Western blot analysis system (Amersham Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer’s protocol was used for detection. The intensity analysis was carried out using SigmaGel 1.0 (Jandel Scientific, San Rafael, CA, U.S.A.). The results are the mean of five independent experiments.

All data are expressed as mean ± S.E.M. The results in each group were compared by one-way analysis of variance (ANONA) followed by Student’s t test. The difference for comparison was considered significant at P<0.05.

Estradiol administration significantly reduced the infarct volume by over 60%, compared with oil-treated control (Fig. 1A and 1B). In particular, the protective effect of estradiol appears significantly in the cerebral cortex. However, estradiol did not protect against injury in the striatum. The ischemic lesion area was 32.6 ± 1.9% and 13.0 ± 1.4% in oil-treated and estradiol-treated animals, respectively. The number of TUNEL positive cells significantly increased in the infarct region (Fig. 2A, 2C, 2D), whereas it was markedly reduced in the cerebral cortex of estradiol-treated animals (Fig. 2B). In the cerebellar cortex, 76.5 ± 6.5% and 2.5 ± 0.9% of cells were TUNEL positive in the ipsilateral cortex of oil-treated and estradiol-treated animals, respectively. In the striatum, 90.5 ± 5.5% and 77.6 ± 3.8% of cells were TUNEL positive in oil-treated and estradiol-treated animals, respectively.

Brain injury induced a decrease of Bcl-2 levels, and estradiol prevented injury-induced down regulation of Bcl-2. The level of Bcl-2 was 0.79 ± 0.08 and 1.02 ± 0.10 in the ipsilateral cortex of oil- and estradiol-treated animals, respectively (Fig. 3). The level of Bax was significantly increased in the ipsilateral cortex of oil-treated animals. In the existence of estradiol, the level of Bax was decreased in the ipsilateral cortex, compared to that of oil-treated animals. The level of Bax was 1.38 ± 0.15 and 1.08 ± 0.13 in the ipsilateral cortex of oil- and estradiol-treated animals, respectively (Fig. 3). The levels of Bcl-x(L) was 1.12 ± 0.15 and 1.28 ± 0.08 in the ipsilateral cortex of oil- and estradiol-treated animals, respectively. Estradiol prevents injury-induced decrease of Bcl-x(L) (Fig. 3).

Previous study demonstrates that the physiological levels of 17β-estradiol protect the brain against ischemic injury by MCAO, and it exerts potent neuroprotective actions in cultured explants of cerebral cortex [15]. Also, estradiol significantly diminished the number of apoptotic nuclei following injury [18]. In this study, our data showed that estradiol treatment significantly reduced the number of apoptotic cells in the cerebral cortex [15]. Also, estradiol significantly protects cerebral cortex from ischemic brain injury. B : The percentage of ischemic lesion area was calculated as lesion area/whole brain section area. Data (n=15) are represented as mean ± S.E.M. * P<0.05 (vs. control).

Fig. 1. Representative photos (A) and infarct volume (B) of brain section from oil- (left panel) and estradiol- (right panel) treated rats prior to MCAO. A : Brain sections were stained by TTC. The ischemic area remained white, while the intact area was stained red. Estradiol treatment significantly protects cerebral cortex from ischemic brain injury. B : The percentage of ischemic lesion area was calculated as lesion area/whole brain section area. Data (n=15) are represented as mean ± S.E.M. * P<0.05 (vs. control).
showed that the level of Bax was significantly increased in ischemic region, and estradiol decreased the level of Bax in the ipsilateral cortex, compared to that of oil-treated animals. More precisely, the level of Bax increases in case of brain injury, and estradiol prevents the injury-induced increase of Bax. In contrast to Bax level, the level of Bcl-2 was decreased in ischemic region, and estradiol prevents the injury-induced decline of Bcl-2. Bcl-2 is an anti-apoptotic factor that inhibits apoptotic cell death, whereas Bax is a pro-apoptotic factor that accelerates apoptotic death in response to a death signal [10]. It is known that a change in the ratio of Bcl-2 to Bax is the critical determinant of cell fate [10]. Our results demonstrated that estradiol prevents cell death through the regulation of Bcl-2 and Bax. Furthermore, Pike et al. [12] demonstrate that estrogen increases the expression of the anti-apoptotic protein Bcl-x(L) in cultured hippocampal neuron. Our results showed that the level of Bcl-x(L) in existence of estradiol coincided with their data. Thus, we suggest the fact that estradiol inhibits cell death by preventing the injury-induced up-regulation of Bax and down-regulation of Bcl-2 and Bcl-x(L). In summary, our findings establish that estradiol plays a potent neuroprotective role in brain injury through the regulation of Bcl-2 family proteins.

ACKNOWLEDGEMENT. This work was supported by the grant from the Korea Science and Engineering Foundation (KOSEF R04–2003–000–10062–0).

REFERENCES