Abstracts of the 2008th Alumni Association Memorial Lectures of the 77th Annual Meeting of the Medical Association of Nippon Medical School

Date: September 5, 2009 Place: Nippon Medical School

Abstracts of the 2008th Alumni Association Medical Research Fund Prize Memorial Lecture (1)

Brain Protection and Regeneration Therapy for Delayed Neuronal Injury in Neurological Disorders

Masayuki Ueda and Yasuo Katayama

Divisions of Neurology, Nephrology and Rheumatology, Department of Internal Medicine, Nippon Medical School

Hippocampal CA1 pyramidal neurons die 3 to 7 days after transient forebrain ischemia in rats and gerbils; this phenomenon is called "delayed neuronal death." Most previous studies of delayed neuronal death examined the effects of ischemia by the duration of occlusion of the common carotid arteries; however, this method sometimes produces variations in CA1 neuronal injury among experimental animals. To improve the reproducibility of CA1 damage, we modified the rat 4-vessel occlusion model, a widely used forebrain ischemia model, using hippocampal depolarization duration as an index for ischemic effect, by monitoring the direct current (DC) potential shift in the hippocampus following common carotid artery occlusion (Fig. 1A: highly controlled rat 4-vessel occlusion model). Figure 1B shows the relationship between hippocampal CA1 neuronal density (7 days after forebrain ischemia) and the ischemic effect assessed by means of the depolarization duration or the occlusion duration of the common carotid arteries. The injury curve for CA1 neurons is sigmoid when assessed on the basis of depolarization duration as an index for ischemic effect but displays considerable variation when assessed on the basis of the occlusion duration (Fig. 1B).

Sublethal ischemia can protect against CA1 neuronal injury induced by a subsequent lethal ischemic insult; this phenomenon is called "ischemic tolerance." We determined the optimal preconditioning insult for induced tolerance using the highly controlled rat 4-vessel occlusion model. Rats were subjected to priming insults of various durations 2 days before test insults of 8 to 9 minutes' depolarization, which can produce almost complete CA1 neuronal loss in naive animals (**Fig. 1B**), and CA1 neurons were counted 7 days after the test insults. **Figure 2A** shows that optimal preconditioning in this model is 2 to 3.5 minutes' depolarization. Many studies have shown a robust and long-lasting CA1 neuron protection by preconditioning; however, the slow progression of cell loss has also been described, raising fundamental concerns regarding the persistence of protection. To address this concern, we examined hippocampi protected by tolerance in long-surviving animals¹. Rats were subjected to preconditioning insults of 2 to 3.5 minutes' depolarization followed by test insults of various durations at 2-day intervals and survived for 1, 2, 4, or 12 weeks after the test insults. **Figure 2B** shows slow

Journal Website (http://www.nms.ac.jp/jnms/)

A: Depolarization monitoring in the hippocampus





B: Injury curve for hippocampal CA1 neurons





injury progression between 1 and 12 weeks' survival in preconditioned hippocampi subjected to test ischemia. Preconditioning 2 days before test insults prolonged the injury threshold evaluated at 1 week's survival; however, the injury threshold markedly regressed (**Fig. 2B**). Thereafter, slight progression of neuronal injury was evident at 12 weeks' survival (**Fig. 2B**). These findings indicate distinct components of lasting and transient protection after ischemic preconditioning, named "true" and "false" preconditioning, respectively. In addition, we found that ischemic depolarization at test insult was delayed in optimally preconditioned hippocampi (**Fig. 3A**). Another set of animals subjected to sham operation, primed insults of 2 to 3.5 minutes' depolarization or no operation (control), followed by test insults at 2-day intervals (**Fig. 3B**). Optimal preconditioning significantly delayed depolarization latency, but not repolarization latency, after common carotid artery occlusion (**Fig. 3B**). Although eliminated as a factor in our study, such delayed depolarization can contribute to protection when the ischemic effect is evaluated on the basis of occlusion time alone ("pseudo" preconditioning). These findings indicate that ischemic tolerance consists of 3 components: "true," "false," and "pseudo" preconditioning. The "false" preconditioning is unique, because CA1 neurons, protected for a short time (1 week after test insult), degrade in an extremely slow manner afterward, as observed in neurological disorders. Therefore, such delayed neuronal injury is important and may be a future therapeutic target.

Recently, bone marrow cell transplantation has attracted considerable attention. Bone marrow contains pluripotent bone marrow stromal cells, which can differentiate into neurons and glial cells. The cells also produce various trophic factors and show a neuroprotective effect against ischemic brain damage. We also examined the effects of transplantation of bone marrow mononuclear cells in an experimental stroke model and found that the larger number of transplanted cells in the brain during the early stage of reperfusion may be important for neuroprotection². On the basis of basic knowledge, we will examine whether bone marrow cell

M. Ueda, et al





Depolarization Latency at Priming Insult (min)





transplantation can inhibit the extremely delayed hippocampal neuron injury found in a highly controlled rat forebrain ischemia model and examine whether transplantation can enhance neuronal regeneration in the hippocampus subjected to ischemia.

References

- 1. Ueda M, Nowak TS Jr: Protective preconditioning by transient global ischemia in the rat: components of delayed injury progression and lasting protection distinguished by comparisons of depolarization thresholds for cell loss at long survival times. J Cereb Blood Flow Metab 2005; 25: 949–958.
- 2. Kamiya N, Ueda M, Igarashi H, et al.: Intra-arterial transplantation of bone marrow mononuclear cells immediately after reperfusion decreases brain injury after focal ischemia in rats. Life Sci 2008; 83: 433–437.