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Short communication

Ischemia-induced changes of platelet endothelial cell adhesion molecule-1 in the hippocampal CA1 region in gerbils

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Abstract

We observed chronological changes of platelet endothelial cell adhesion molecule-1 (PECAM-1), final mediator of neutrophil transendothelial migration, immunoreactivity, and protein level in the gerbil hippocampus proper after 5 min of transient ischemia. One day after ischemic insult, PECAM-1 immunoreactivity and protein level increased slightly in the hippocampus proper. Thereafter, PECAM-1 immunoreactivity and protein level increased significantly in the hippocampus proper by 4 days after ischemic insult. Especially, PECAM-1 in the hippocampal CA1 region was higher than that in the CA2/3 region. Five days after ischemic insult, PECAM-1 immunoreactivity decreased compared to the 4 days post-ischemic group. However, the RNA levels of PECAM-1 in the hippocampus proper were significantly decreased in the 4 days post-ischemic groups compared to that in the sham-operated group. This result suggests that the increase of PECAM-1 and decrease of PECAM-1 RNA in the CA1 region 4 days after ischemia may be associated with transmigration of neurotrophil.

Theme: Disorders of the nervous system
Topic: Ischemia

Keywords: Transient forebrain ischemia; Hippocampus proper; CA1 region; Delayed neuronal death; Microvessels; Platelet endothelial cell adhesion molecule-1; Gerbil

The physiological barrier between the blood and the central nervous system (CNS), the so-called blood–brain barrier (BBB), is composed of brain parenchymal microvascular endothelial cells, ensheathed by astrocyte foot processes and pericytes. This barrier exhibits a low and strictly controlled permeability to blood-borne compounds, mainly due to the presence of interendothelial tight junctions [9]. However, during inflammatory situations, massive infiltration of leukocytes is observed in the brain tissue, together with the expression of various adhesion molecules such as selectins and immunoglobulin superfamily on the surface of cerebral vascular endothelium [2]. Inflammation is a hallmark of various CNS diseases such as bacterial and viral infections, multiple sclerosis, Alzheimer's disease, and cerebral ischemia.

Transient forebrain ischemia, produced by prolonged deprivation of blood flow to the brain, results in the insidious delayed neuronal degeneration of specific vulnerable neurons within the CA1 region of the hippocampus [7,8,14,18]. Expansion of infarction after ischemia may be due to microcirculatory disturbance [3] and selective neuronal vulnerability [6]. The microcirculatory disturbance may be induced by forming capillary block, BBB damage,

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vasoconstriction, biochemical mediators such as reactive oxygen species, leukotrienes, cytokines, platelet-activating factor, and proteolytic enzymes stemming from interaction between leukocytes and perturbed endothelium in the ischemic area [3,17].

It has been reported that platelet endothelial cell adhesion molecule-1 (PECAM-1, CD-31), a member of the immunoglobulin-associated cell adhesion molecule family, is present on neutrophils and endothelial cells and mediates the final common pathway of neutrophil transendothelial migration [12]. However, up to date, no study has evaluated the chronological changes of PECAM-1 in the hippocampus after transient forebrain ischemia. Therefore, in the present study, we investigated the ischemia-induced changes of PECAM-1-immunoreactivity and protein level in the hippocampus after 5 min of transient forebrain ischemia to examine relationship between the delayed neuronal death and PECAM-1 in the hippocampus proper in the gerbil.

Male Mongolian gerbils (*Meriones unguiculatus*) weighing 66–75 g were placed under general anesthesia with a mixture of 2.5% isoflurane in 33% oxygen and 67% nitrous oxide. A midline ventral incision was made in the neck. Both common carotid arteries were isolated, freed of nerve fibers, and occluded using non-traumatic aneurysm clips. Complete interruption of blood flow was confirmed by observing the central artery in eyeballs using an ophthalmoscope. After 5 min of occlusion, the aneurysm clips were removed from both of the common carotid arteries. Restoration of blood flow (reperfusion) was observed directly under the ophthalmoscope. Sham-operated animals were subjected to the same surgical procedures except that common carotid arteries were not occluded. Body temperature was monitored and maintained at 37 ± 0.5 °C during the surgery and during the immediate postoperative period until the animals recovered fully from anesthesia. Sham-operated animals served as controls (n = 20). At the designated times (30 min, 3 h, 12 h, 1 day, 2 days, 4 days, and 5 days after the surgery), the sham-operated and operated animals (n = 20 at each time point) were sacrificed for this study [7,18].

Ten animals in each group were used in histochemical and immunohistochemical studies. Animals were anesthetized with pentobarbital sodium and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in 0.1 M PB (pH 7.4) at the designated times after the surgery. Brains were removed and postfixed in the same fixative for 6 h.

Three animals in each group were used for delayed neuronal death in the hippocampal CA1 region. The brain tissues were dehydrated by placing fixed brain tissues successively for 2 h in 50%, 70%, 80%, 90%, 95%, and 100% ethanol bathes at room temperature. Then, the tissues were placed 2 times, each time for 1 h, in fresh pure xylene and then in molds containing melted paraffin (Histowax, Leica). Serial transverse sections of 7 μm thickness were cut, and the sections were mounted on gelatin-coated microscopic slides. Cresyl violet acetate (Sigma, MO) was dissolved at 1.0% (w/v) in distilled water, and glacial acetic acid was added in this solution. Before and after staining for 2 min at room temperature, the sections were washed twice in distilled water. After dehydration, the sections were mounted in Canada Balsam (Kato, Japan).

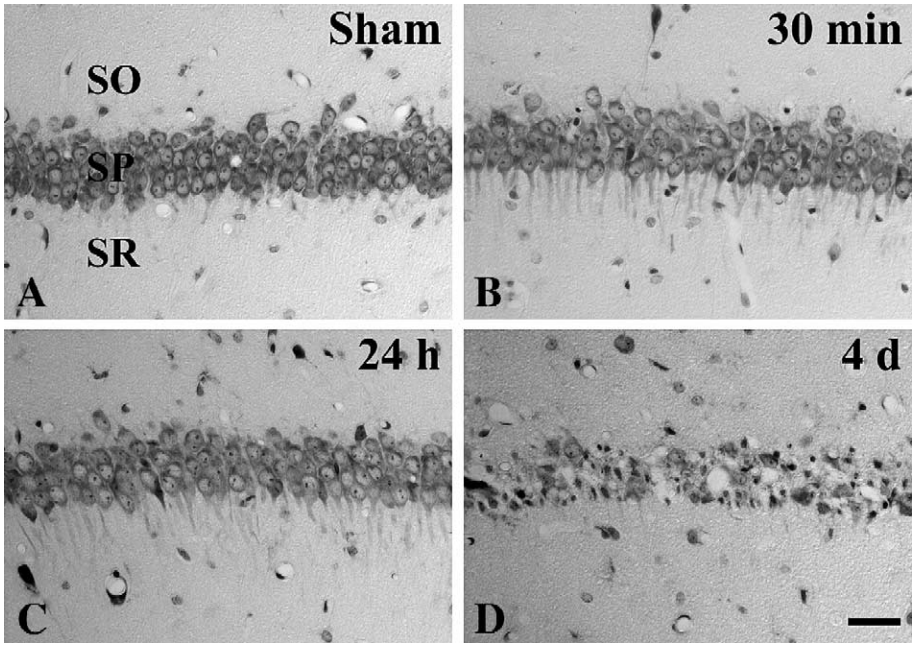

Fig. 1. The cresyl violet staining of the hippocampal CA1 region in the sham-operated group (A) at 30 min (B), 24 h (C), and 4 days (D) after ischemia–reperfusion. CA1 pyramidal cells in the stratum pyramidale are well stained with cresyl violet in the sham-operated group (A). There is no finding of delayed neuronal death in the CA1 pyramidal cells by a few days after ischemic insult (B, C), while the delayed neuronal death happens in the stratum pyramidale of the CA1 region 4 days after ischemic insult (D). Scale bars = 100 μm.