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Short communication

Calbindin D-28k is expressed in the microvascular basal lamina in the ventral horn at early time after transient spinal cord ischemia in the rabbit

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Abstract

Much evidence has been accumulated that the increased expression of calbindin D-28k (CB) is involved in the blockade of calcium-evoked excitotoxicity in cerebral ischemia. We investigated the expression of CB in the basal lamina of microvessels in the ventral horn of the rabbit spinal cord after transient spinal cord ischemia. Spinal cord sections at the level of L_7 were immunostained using monoclonal antibody raised against CB at light and electron microscopic levels. CB immunoreactivity was detected in the basal lamina of microvessels at 30 min after ischemic insult. By 3 h after ischemia, CB immunoreactivity was increased in the basal lamina of the microvessels. CB immunoreactivity began to decrease at 6 h after ischemia and nearly disappeared at 48 h after ischemic insult. For calcium detection in the blood vessels of spinal cord, we conducted an alizarin red staining. Alizarin red reactivity was detected in some microvessels at 3 h after ischemic insult. Our results suggest that the ectopic expression of CB in the microvascular basal laminae may be associated with the buffering of calcium in the endothelial cells of microvessels after ischemic damage.

Theme: Disorders of the nervous system
Topic: Ischemia

Keywords: Transient spinal cord ischemia; Calcium; Microvessels; Basal lamina; Calbindin D-28k; Immunohistochemistry; Electron microscopy; Rabbit

Spinal cord ischemia resulting from aortic occlusion may evoke neuronal degeneration of spinal cord neurons and bring about loss of motor function [31]. Spinal motor neurons are more vulnerable to ischemia than sensory neurons in the dorsal horn. The rabbit of aortic occlusion has been one of the most frequently used models for the study of transient spinal cord ischemia because segmental branches of the abdominal aorta to supply lumbosacral segments of the spinal cord have not collateral anastomosis [31].

Excitotoxicity is related to the neuronal damage resulting from excessive activation of excitatory amino acid [7,28]. Exact mechanism of excitotoxicity is not fully understood, but it is clear that the influx of Ca^{2+} into neurons plays important roles in the excitotoxic neuronal damage [3]. The enhanced Ca^{2+} entry and loss of calcium homeostasis that occur during the reperfusion period following ischemia could account for neuronal vulnerability [24]. An upturn of intracellular calcium levels following ischemia has been

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supported to the hypothesis that calcium overload may play a pivotal role in post-ischemic cell death [2,6,14], although other authors interpret this process as a secondary phenomenon accompanying another irreversible mechanism of neuronal damage [9,27]. According to the calcium hypothesis, the pathological level of intracellular calcium activates biochemical processes leading to enzymatic breakdown of proteins and lipids, to malfunctioning of mitochondria, energy failure, and ultimately to cell death [3,28].

Calbindin D-28k (CB) is one of the calcium binding proteins, which act as a calcium transporter and an intercellular free calcium buffer. These proteins promote or restrict calcium-dependent events in the cellular metabolism including axonal transport [21] and the release of the neurotransmitters [22]. CB has been extensively studied in CNS diseases such as epileptic seizures [19], Alzheimer's disease [29], and transient forebrain ischemia [17]. This phenomenon is implicated in disturbance of calcium homeostasis. These reports put forward that the expression of CB may be associated with resistance of neuronal subpopulations to degeneration.

Blood–brain barrier (BBB) disruption plays a critical role in the pathophysiology of ischemia–reperfusion [11]. In the pathological condition of ischemia–reperfusion, the digestion of endothelial basal lamina occurs as early as 2 h after ischemia–reperfusion [13], which may make BBB permeable within a few hours after ischemia [4]. It has been reported that an increase in intracellular calcium was observed in human aortic endothelial cells exposed to hypoxia. However, there were few studies on the correlation between calcium and spinal cord ischemia [16,30]. Therefore, the purpose of this study is to observe whether CB immunoreactivity appears or not in microvessels in the ventral horn after transient spinal cord ischemia at light and electron microscopic levels.

We used male white rabbits (2.5–3.0 kg) obtained from the Experimental Animal Center, Hallym University, Chunchon, South Korea. The animals were housed at constant temperature (22 ± 2 °C) and relative humidity (55 ± 5%) with the fixed 12 h light/dark cycle and free access to food and water. Procedures involving animals and their care were conformed by the institutional guidelines, which are in compliance with current international laws and policies (NIH *Guide for the Care and Use of Laboratory Animals*, NIH Publication No. 85-23, 1985). All experiment was conducted to minimize the number of animals used and suffering.

The animals were placed under general anesthesia with the mixture of 2.5% isoflurane in 30% oxygen and 70% nitrous oxide. A ventral midline incision was made in the abdomen. Abdominal aorta was isolated underneath the left renal artery and then occluded using nontraumatic aneurysm clip. After 15 min of occlusion, the aneurysm clip was removed, and restoration of blood flow (reperfusion) was confirmed at abdominal aortic artery. Sham-operated controls (n = 15) were subjected to the same surgical procedures except that abdominal aortic artery was not occluded. Body temperature was monitored and maintained at 38.7 °C ± 0.3 °C during surgery and the immediate postoperative period until the animals fully recovered from anesthesia. At designated times (0.5, 1, 3, 6, 12, 24, and

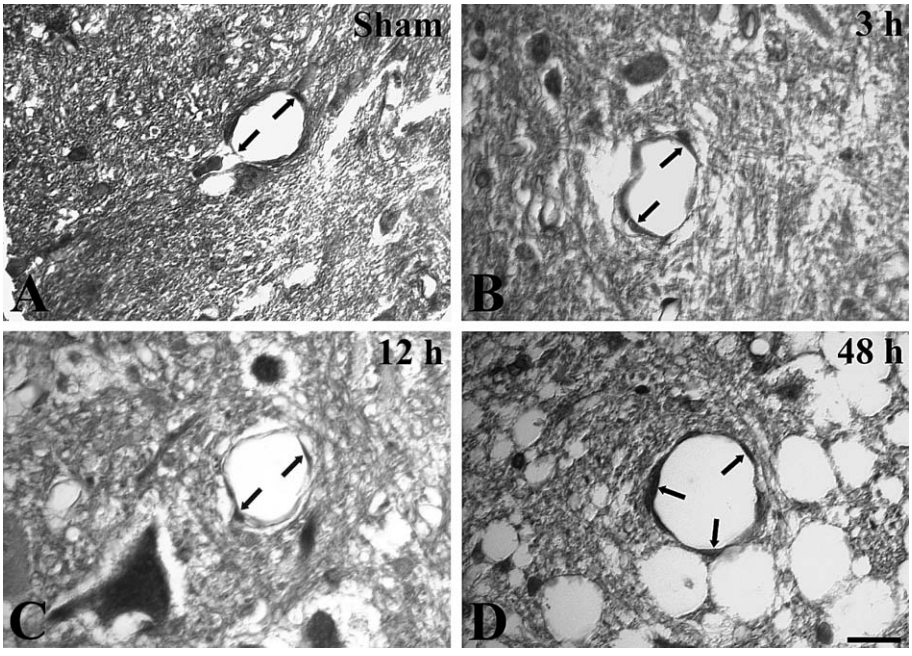

Fig. 1. The photomicrographs of alizarin red reactivity in the ventral horn of the spinal cord after 15 min of ischemic insult. Alizarin red reactivity is not detectable on the blood vessel (arrows) in the ventral horn of the sham-operated rabbit (A). At 3 h after ischemia–reperfusion (B), alizarin red reactivity is observed on endothelial cells of microvessel (arrows). At 12 h after ischemia–reperfusion (C), alizarin red reactivity is detected in some neurons and endothelial cells in ventral horn of spinal cord. At 48 h after ischemia–reperfusion (D), alizarin red reactivity is observed in the ventral horn of the spinal cord. Scale bar = 25 μm.