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Research report

Early immature neuronal death is partially involved in memory impairment induced by cerebral ischemia

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HIGHLIGHTS

- We found early immature neuronal death, which was determined by DCX and NeuN-double-staining.
- Injection of caspase-3 inhibitor rescued cells from immature neuronal death.
- z-DEVD-fmk treatment partially rescued ischemia-induced spatial memory impairment.
- Ischemia-induced LTP impairment in the perforant pathway was restored by z-DEVD-fmk treatment.

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ABSTRACT

Memory impairment is a common after an ischemic stroke. While delayed neuronal death in the CA1 region is usually linked to cerebral ischemia-induced memory impairment, the role of early immature neuronal death within the DG region in the memory state of an ischemic stroke model has rarely been studied. Here, we show a partial role of immature neuronal death in memory impairment in a global ischemia model. We found early immature neuronal death, which was determined by DCX and NeuN-double-staining. Injection of z-DEVD-fmk, a caspase-3 inhibitor, into the DG region rescued cells from immature neuronal death in the DG region without affecting delayed neuronal death in the CA1 region of an ischemic brain. Moreover, z-DEVD-fmk treatment partially rescued ischemia-induced spatial memory impairment. We also found that ischemia-induced LTP impairment in the perforant pathway was restored by z-DEVD-fmk treatment. These results suggest that early immature neuronal death is partially involved in ischemia-induced spatial memory impairment.

1. Introduction

Vascular dementia is the second most common form of dementia in older adults [1]. Vascular dementia is caused by problems in the supply of blood to the brain, which is typically caused by a series of ischemic strokes [2]. Low blood supply damages vulnerable structures including the hippocampus [3,4]. The CA1 of the hippocampus has been reported as the most vulnerable region, and delayed neuronal death occurrs in this area [3]. Therefore, the damage to the CA1 region is thought to cause other neurological

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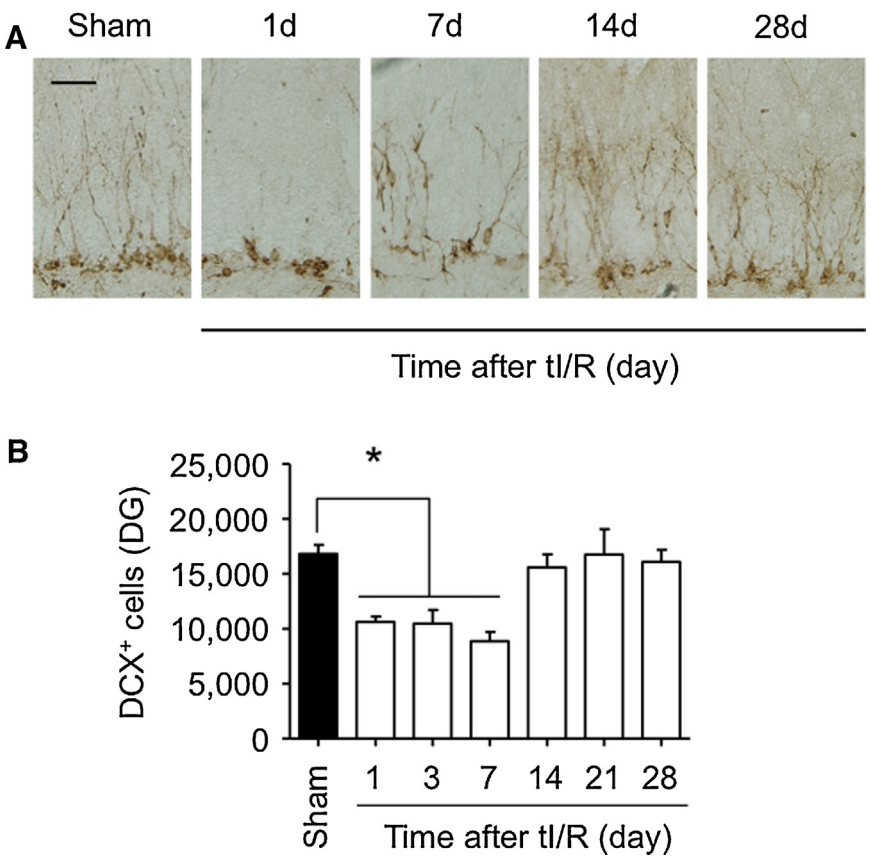

Fig. 1. The effect of tI/R on DCX immunoreactivity in the hippocampal DG region. Mice were subjected tI/R and sacrificed at designated time points (1, 3, 7, 14, 21, and 28 days after tI/R). And immunohistochemistry with anti-DCX antibody was conducted. (A) Representative photograph of DCX-positive cells. Bar = 25 μm. (B) Quantitative analysis of the number of DCX-positive cells in the DG region is represented. Data are represented as mean ± SEM. n = 5/group. $^{*}P < 0.05$.

symptoms, such as memory impairment, which are observed in the ischemic stroke model [4]. Various neuroprotective drugs exerting protective effects on CA1 neuronal death have been discovered to avoid memory impairment [5–7]. While recent reports indicate that other regions including the dentate gyrus (DG) are also vulnerable to ischemic insults, the role of these regions in the symptoms in the ischemic stroke model are still not well understood [8,9].

Although there is still controversy [10], hippocampal neurogenesis is considered important for spatial pattern separation, which is a formation of distinct representations of similar inputs [11], updating and strengthening of spatial memory [12,13]. Immature neurons are believed to be important for this effect of neurogenesis in pattern separation [10,11] and spatial memory [14–17]. Therefore, the changes in these signaling and immature neuronal fates may influence spatial memory strengthening.

Recently, several studies including ours have reported that immature neurons died at the early ischemic period in the DG region [8,9]. In our previous study, we found that early treatment of DEVD-fmk rescued early immature neuronal death and blocked delayed facilitation of neural proliferation by transient forebrain ischemia [8]. Based on the role of immature neurons on spatial memory and pattern separation, we hypothesized that this early immature neuronal death may be involved in memory impairment, and rescue of this early event by DEVD-fmk may block memory impairment in the ischemic stroke model. However, the role of early immature neuronal death in the DG region on ischemia-induced memory impairment has not been studied before. In the present study, we found that early immature neuronal death is partially involved in ischemic insults-induced spatial memory impairment.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice (25–28 g, 10 weeks) were purchased from the Orient Co., Ltd., a branch of Charles River Laboratories (Seoul). Animals were housed four cage and allowed access to water and food *ad libitum*. The cages were maintained at a constant temperature (23 ± 1 °C) and relative humidity (60 ± 10%) under a 12-h light/dark cycle (lights on from 07:30 to 19:30). All experimental protocol for animals was approved by the Institutional Animal Care and Use Committee of the Kyung Hee University, Korea. The experimental animal protocols were approved by the Institutional Animal Care and Use Committee of Kyung Hee University, Korea (approved No. KHP 2010-10-14). All animals were anesthesized with Zoletil 50 (1 mg/kg, i.m.) for surgery and sacrifice. All efforts were made to minimize suffering.

2.2. Microinfusion of drugs

Cannula implantation was conducted as described in our previous study [18]. All mice were implanted with stainless-steel guide cannulae (Plastics One, Roanoke, VA) aimed at the dorsal third ventricle. Mice were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) under Zoletil 50® anesthesia (10 mg/kg, i.m.), and guide cannulae (26 G) were aimed at the dorsal third ventricle (stereotaxic coordinates: AP, −1.90 mm; DV, −2.50 mm) using an atlas of the mouse brain [19]. The guide cannulae were fixed to the skull with dental cement that also served to close the wound and covered with dummy cannulae. Following surgery, mice were