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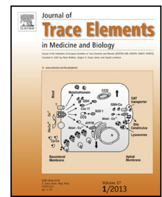

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Zinc chelation reduces traumatic brain injury-induced neurogenesis in the subgranular zone of the hippocampal dentate gyrus

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ABSTRACT

Numerous studies have demonstrated that traumatic brain injury (TBI) increases hippocampal neurogenesis in the rodent brain. However, the mechanisms underlying increased neurogenesis after TBI remain unknown. Continuous neurogenesis occurs in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) in the adult brain. The mechanism that maintains active neurogenesis in the hippocampal area is not known. A high level of vesicular zinc is localized in the presynaptic terminals of the SGZ (mossy fiber). The mossy fiber of dentate granular cells contains high levels of chelatable zinc in their terminal vesicles, which can be released into the extracellular space during neuronal activity. Previously, our lab presented findings indicating that a possible correlation may exist between synaptic zinc localization and high rates of neurogenesis in this area after hypoglycemia or epilepsy. Using a weight drop animal model to mimic human TBI, we tested our hypothesis that zinc plays a key role in modulating hippocampal neurogenesis after TBI. Thus, we injected a zinc chelator, clioquinol (CQ, 30 mg/kg), into the intraperitoneal space to reduce brain zinc availability twice per day for 1 week. Neuronal death was evaluated with Fluoro Jade-B and NeuN staining to determine whether CQ has neuroprotective effects after TBI. The number of degenerating neurons (FJB (+)) and live neurons (NeuN (+)) was similar in vehicle and in CQ-treated rats at 1 week after TBI. Neurogenesis was evaluated using BrdU, Ki67 and doublecortin (DCX) immunostaining 1 week after TBI. The number of BrdU, Ki67 and DCX positive cell was increased after TBI. However, the number of BrdU, Ki67 and DCX positive cells was significantly decreased by CQ treatment. The present study shows that zinc chelation did not prevent neurodegeneration but did reduce TBI-induced progenitor cell proliferation and neurogenesis. Therefore, this study suggests that zinc has an essential role for modulating hippocampal neurogenesis after TBI.

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Introduction

Despite the longstanding notion that the adult brain does not produce new neurons, it has recently been demonstrated that throughout the lifetime of mammals, newly generated neurons are born within the subgranular zone (SGZ) of the dentate gyrus and migrate a short distance into the granular cell layer [1,2]. Once there, these newly born cells become functionally integrated into existing hippocampal circuitry by sending dendrites into the molecular layer and axons into the CA3 region [3–5]. The full functional implications of this continuous morphological and structural rearrangement is not known, however it may provide a basis for network plasticity for hippocampus-dependent learning and memory. Traumatic brain injury (TBI) increases adult neurogenesis in the subgranular zones (SGZ) of dentate gyrus of hippocampus in rodents and in the human brain. TBI-related injury to this structure has been associated with learning and memory deficits. Recent observations that hippocampal neurogenesis is implicated in learning and memory functions [6] has raised hopes that injury-induced neurogenesis may represent an endogenous repair mechanism or otherwise serve as a basis of therapeutic intervention. Indeed, promising studies have demonstrated that neural progenitors migrate to injured brain regions and differentiate into the neuronal phenotype specific to the area [7–12]. Kleindienst et al. [13] showed the presence of a proliferative response that peaks during the first week after TBI, and a return to baseline levels of proliferation in the dentate gyrus by 35 days after injury. However, the exact mechanisms that regulate progenitor cell proliferation and neurogenesis response to TBI are not well understood.

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It has previously been demonstrated that hypoglycemia increases the number of proliferating progenitor cells and immature neurons in the SGZ of rats. However, 4 weeks post-injury, this initial increase is predictably followed by a sustained decline of progenitor cell proliferation and immature neurons [14]. The mechanism underlying the rise and decline of hippocampal progenitor cell proliferation after hypoglycemia is unclear. However, we have proposed that synaptic zinc release from mossy fiber is a key factor in this process. According to this hypothesis, a pathologically large release of synaptic zinc occurs after hypoglycemia, which stimulates neurogenesis at short latency post-insult, but is subsequently depleted, leading to a decline in neurogenesis due to reduced zinc release or a reduced concentration of vesicular zinc [15].

Zinc is an essential component of more than 1000 enzymes and is critical for regulation of a multitude of cellular processes, including cell division and DNA synthesis [16]. Specifically, zinc is involved in hormonal regulation of cell division in cells regulated by insulin-like growth factor-I (IGF-I) [16] or nerve growth factor (NGF) [17]. Divalent zinc is the second most abundant transition metal in the brain following iron. Chelatable zinc is highly localized in the synaptic vesicle of mossy fiber of the dentate granule cell [18,19], sites where neurogenesis and neural migration are most active in the adult brain [20]. Zinc has long been recognized as a biologically essential element for brain function [21–23]. Division and migration of cerebellar granular cells are reduced following a period of severe zinc deficiency [24,25]. Golub et al. [26] showed that zinc deficiency impaired performance in short-term-memory tasks. Recent studies demonstrated that age-related decline of spatial memory, impaired spatial memory and impaired behavior were found in ZnT3 knock-out mice [27–29]. Thus, zinc appears to be an essential element required for cell division, proliferation, migration and development, and further suggests that this element may play a critical role in neurogenesis and cognitive function.

Clioquinol (5-chloro-7-iodo-8-hydroxy-quinoline, CQ) is a metal chelator. Studies on the effects of endogenous zinc under physiological and pathological conditions have exploited chelating agents to elucidate the specific role played by this ion [30–32]. Recent animal studies have demonstrated that CQ decreased basal neurogenesis as well as on seizure-induced transient hippocampal neurogenesis [33].

In the present study, we aimed to elucidate the potential role of brain zinc in modulating hippocampal neurogenesis after TBI by using a membrane-permeable zinc chelator, CQ.

Materials and method

Experimental animals

The care and handling of animals were in accordance with institutional guidelines and were approved by the Institutional Animal Studies Committee of Hallym University in Chuncheon, Korea (protocol # Hallym 2011-67-1). In this study, we used 8 weeks old male Sprague–Dawley rats (250–300 g; DBL Co., Korea). The animals were housed in a temperature- and humidity-controlled environment (22 ± 2 °C, 55 ± 5% and a 12 h light:12 h dark cycle), and supplied with Purina diet (Purina, Gyeonggi, Korea) and water *ad libitum*.

Weight drop model

Rats were deeply anesthetized with 2% isoflurane and a 75:25 mixture of nitrous oxide/oxygen. Rats were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). The scalp and temporalis muscles were reflected, and a 5.0 mm diameter hole was drilled through the skull (3.0 mm lateral to the midline and 4.0 mm lambda to the bregma) [34–37]. TBI was performed using an EM controlled impact device (Impact One^{TM} Stereotaxic Impactor, Richmond, IL). For the mechanical trauma, a 5 mm blunt steel impactor tip was retracted and positioned above intact dura. The injury was triggered using the myNeuroLab controller at a strike velocity of 4.5 m/s, strike depth of 2.5 mm, and dwell time of 500 ms [38]. All rats were maintained at a core temperature of 36–37.5 °C during and after surgery, until ambulatory. Rats with seizures were excluded from data analysis ($n = 1$).

Clioquinol administration

To deplete levels of vesicular zinc or to chelate extracellular zinc, a zinc chelator, clioquinol was used. Rats were injected with clioquinol (CQ, 30 mg/kg, i.p.) twice per day (9–10 AM and 5–6 PM) for 1 week after TBI or without TBI. Clioquinol was dissolved with dimethyl sulfoxide (1% DMSO, Sigma). In the TBI-experienced rats, CQ injection was started at 24 h after TBI. Control rats were injected with the same volume of DMSO. The non-TBI group also had CQ/DMSO or DMSO vehicle only.

Neuron death

Neuronal death was evaluated 1 week after TBI. Rats were intracardially perfused with 0.9% saline followed by 4% paraformaldehyde (PFA). The brains were post-fixed with 4% PFA for 1 h and then incubated with 30% sucrose for cryo-protection. Thereafter, the entire brain was frozen and sectioned with a cryostat microtome at 30 μm thickness and cryoprotection solution. Brain sections were stained for the Fluoro-Jade B staining (FJB) [39,40]. Degenerating neurons were detected with 450–490 nm excitation and a 515 nm emission filter. To quantify neuronal death, sections were collected from 3.2 mm to 4.5 mm posterior to bregma and five coronal sections were analyzed from each animal. These sections were then coded and given to a blinded experimenter who counted the number of degenerating neurons in the hippocampal CA1 and dentate gyrus (DG).

Detection of live neurons

To identify neuroprotective effects of CQ after TBI, brain sections were immunohistochemically stained by NeuN. Monoclonal anti-NeuN, clone A60 antibody (diluted 1:100, Millipore Co., Billerica, MA, USA) was used as the primary antibody in PBS containing 0.3% Triton X-100 overnight at 4 °C. The sections were washed three times for 10 min with PBS, incubated in biotinylated anti-mouse IgG (Vector, Burlingame, CA, USA) and ABC complex (Vector, Burlingame, CA, USA), diluted 1:250 in the same solution as the primary antiserum. Between the incubations, the tissues were washed with PBS three times for 10 min each. The immune reaction was visualized with 3,3-diaminobenzidine (DAB, Sigma–Aldrich Co., St. Louis, MO, USA) in 0.01 M PBS and the tissues were mounted on the gelatin-coated slides. The immunoreactions were observed under the Olympus IX70 inverted microscope (Olympus Co., Shinjuku, Tokyo, Japan).

BrdU labeling

To test the effects of zinc chelation on neurogenesis, BrdU was injected twice daily for 4 consecutive days starting 24 h after TBI [12]. The thymidine analog BrdU was administered intraperitoneally (50 mg/kg; Sigma, St. Louis, MO, USA) to investigate the progenitor cell proliferation. The rats were killed 1 week after TBI.

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