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MILD HYPOTHERMIA FACILITATES THE LONG-TERM SURVIVAL OF NEWBORN CELLS IN THE DENTATE GYRUS AFTER TRAUMATIC BRAIN INJURY BY DIMINISHING A PRO-APOPTOTIC MICROENVIRONMENT

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Abstract—Although previous research has demonstrated that traumatic brain injury (TBI) accelerates the proliferation of neural stem cells in dentate gyrus of the hippocampus, most of these newborn cells undergo apoptosis in a traumatic microenvironment. Thus, promoting the long-term survival of newborn cells during neurogenesis is a compelling goal for the treatment of TBI. In this study, we investigated whether mild hypothermia (MHT) therapy, which mitigates the multiple secondary injury cascades of TBI, enhances the survival of newborn cells. SD rats were subjected to unilateral fluid percussion injury and received MHT therapy for 4 h (33.5 °C). Bromodeoxyuridine (BrdU) was administered to label the mitotic cells. Spatial learning and memory were evaluated with the Morris water maze test. Brain sections were immunostained with antibodies against BrdU, DCX (a neuroblast marker) or NeuN (a mature neuron marker). The apoptosis levels in the dentate gyrus were examined with antibodies against the apoptotic proteins FAS, FASL, Bcl-2 and cleaved caspase 3. The results indicated that MHT could significantly prevent TBI-induced cognitive impairments. At 1 week after injury, the density of BrdU-immunoreactive cells significantly increased in both TBI and TBI + MHT rats. At 4 weeks after injury, the density of BrdU-positive cells further increased in TBI + MHT rats, whereas the density declined in the TBI rats. The density of DCX-positive cells in SGZ of the hippocampus at 1 week after injury in the TBI + MHT rats was significantly greater than in the TBI rats. Moreover, the density of NeuN-positive cells in the subgranular zone at 4 weeks after injury and in the granule cell layer at 7 weeks after injury was significantly increased in the TBI + MHT rats. The TBI + MHT rats displayed a lower level of apoptosis in the dentate gyrus compared with the TBI rats. These data indicate that TBI could only facilitate a burst of proliferation and short-term survival of newborn cells, whereas TBI + MHT could facilitate long-term survival and maturation of newborn cells through diminishing pro-apoptotic microenvironment. These results suggest that MHT-mediated neurogenesis may have an important therapeutic potential for the endogenous repair of TBI.

Key words: traumatic brain injury, mild hypothermia, survival, neurogenesis, apoptosis.

INTRODUCTION

Traumatic brain injury (TBI) is the leading cause of mortality and morbidity, particularly among the young, and constitutes a major public health problem throughout the world. TBI leads to cellular dysfunction and death and results in a variety of neurological impairments such as cognitive deficits. To date, no effective treatment has been successfully translated to the clinical setting except for routine medical intervention and care. Neurogenesis occurs in the two neurogenic regions of adult mammalian brains: the subgranular zone (SGZ) in the dentate gyrus (DG) of the hippocampus and the subventricular zone in the forebrain, in which neural stem cells (NSCs) reside. Neurogenesis from NSCs may be a promising resource for the prevention of cognitive or even other neurological impairments after TBI (Deng et al., 2010).

Neurogenesis in the adult hippocampus is generally limited under normal physiological conditions, but it could be induced after injury (Gould, 2007). It is becoming increasingly clear that TBI can stimulate NSC proliferation in the DG of the adult hippocampus (Dash et al., 2001). However, it remains controversial whether these newborn cells could survive and differentiate into mature neurons (Richardson et al., 2007; Yu et al., 2008). There are conflicting reports about neurogenesis in the DG. Some studies have reported that neurogenesis increases after TBI (Chen et al., 2003), some have reported no changes (Chirumamilla et al., 2002; Rice et al., 2003), whereas some have even reported decreases in neurogenesis after TBI (Braun et al., 2002; Rola et al., 2006). The development of newborn cells can be influenced by interactions within the local milieu and the niche (Zhao et al., 2008;

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Ming and Song, 2011). Even in physiological environments, only a subset of newborn cells survives and differentiates into mature neurons, whereas the majority undergoes programed cell death. Under pathological conditions, there may be a variety of oxygen free radicals or inflammatory factors present post-TBI, and more newborn cells may suffer apoptosis, and these factors may explain why neurogenesis does not increase after TBI. Therefore, facilitating the survival of newborn cells during adult neurogenesis is a particularly compelling target process for the endogenous repair of TBI. Additional intervention is required to enhance neurogenesis and prevent neurological deficits after TBI.

Accumulating evidence demonstrates that mild hypothermia (MHT) has neuroprotective effects against TBI (Faridar et al., 2011) and plays an active role in improving the microenvironment of the injured hippocampus by preventing multiple, specific secondary injury mechanisms. The mechanisms prevented include the accumulation of lactic acid, the production of free radicals, the production of excitotoxic neurotransmitters, and pro-inflammatory and apoptotic pathways (Miyazawa et al., 2003; Olsen et al., 2003; van der Worp et al., 2007; Choi et al., 2012). In order to successfully repair damage to the brain caused by TBI, additional events are required to increase not only the proliferation of NSCs, but to also prevent newborn cells from dying. Thus, we aimed to study the role of mild hypothermia on the long-term survival of newborn cells in the DG of the hippocampus after TBI and its possible mechanisms.

EXPERIMENTAL PROCEDURES

Antibodies and chemicals

Mouse monoclonal antibody (mAb) against 5-bromo-2-deoxyuridine (BrdU) (1:100) was obtained from Millipore (Billerica, MA, USA). Rabbit polyclonal antibodies (pAbs) against doublecortin (DCX) (1:100), NeuN (1:200), caspase 3 (1:200), cleaved caspase 3 (1:200), FAS (1:50), FASL (1:100) and mAb against Bcl-2 (1:200) were obtained from Abcam (Cambridge, UK). BrdU, Hoechst and Bicinchoninic Acid (BCA) kit were obtained from Sigma (St. Louis, MO, USA). Peroxidase-conjugated goat anti-rabbit (1:200) and goat anti-mouse secondary antibodies (1:200) were obtained from Pierce (Rockford, IL, USA). Alexa Fluor 568-conjugated (1:1000) and Oregon Green 488-conjugated secondary antibodies (1:1000) were from Invitrogen (Carlsbad, CA, USA).

TBI model and mild hypothermia therapy

Eight-week male SD rats were purchased from the Chinese Military Academy of Medical Sciences and maintained in the animal center of the Key Laboratory of Neurotrauma Repair in Tianjin, China. All animal experiments were performed according to the ‘‘Policies on the Use of Animals and Humans in Neuroscience Research”, which was revised and approved by the Society for Neuroscience in 1995.

Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium solution at a dose of 50-mg/kg body weight and were surgically prepared for lateral fluid percussion brain injury or sham operation as described (Tu et al., 2012). Briefly, a 4-mm craniotomy overlying the right parietal cortex (3.6 mm posterior to the bregma and 2.5 mm lateral to the midline) was performed. A plastic injury tube was then placed over the exposed dura, bonded by adhesive and dental acrylic and then allowed to harden. Rats were attached to the fluid percussion injury device (Custom Design and Fabrication, VA, USA) via a female Luer-Lok adapter. Severe brain injury was then induced by the rapid injection of saline solution using a pressure pulse (2.0 atm) into the closed cranial cavity. Rats were randomly assigned into three groups with different treatment strategies, namely the sham, TBI, and TBI + MHT groups. Sham rats were subjected to craniotomies, but did not receive fluid percussion injuries. Rats in TBI + MHT group were cooled by an ice blanket machine after injury. Body and brain temperatures were measured using a rectal thermometer and a thermistor in the temporalis muscle, respectively. A target rectal temperature of 33.5 °C was achieved within 15 min. A constant rectal temperature range of 33.3–33.8 °C was maintained for 4 h using an ice blanket machine and an automatized-feedback heat-lamp system. The temperature was then rewarmed naturally. Normothermic rats were kept at a constant temperature (37 °C) with an automatized heating pad connected to a rectal temperature probe. BrdU (10 mg/100 g) was injected either right after injury, or 1, 2, 3, 4 or 5 days after injury.

Morris water maze test

The Morris water maze test was performed according to the procedure described previously (Chen et al., 2014). Briefly, the rats were trained to find a submerged platform by using a stationary array of cues outside the pool tub. The water was made opaque by using black ink for chiaroscuro. Four spaced trials a day were performed from 21 d to 26 d after TBI. The probe tests were performed with the platform 26 d after TBI and without the platform 28 d after TBI. The swimming paths during the probe test were monitored using an automatic tracking system. This system was used to record the swimming paths and the time spent in each quadrant.

Immunofluorescence

The animals were deeply anesthetized and then transcardially perfused with 100 ml saline solution, followed by 400 ml 4% paraformaldehyde (PFA) solution. The brains were removed and post-fixed overnight in PFA. Coronal brain sections were cut with a thickness of 30 μm using a vibrating microtome (Leica VT1000 S, Germany). The sections were permeabilized in 0.5% Triton X-100 in PBS. The sections were then immersed in 0.5% H_2O_2 in methanol for 10 min to block endogenous peroxidases and non-specific binding sites were blocked with 5% non-fat milk in PBS for 1 h at room temperature. Afterward, the sections were incubated with primary