Brain Research 1669 (2017) 141–149

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/bres

Research report

Establishment of an ideal time window model in hypothermic-targeted temperature management after traumatic brain injury in rats

Wan-Yong Zhao [a,1], Shao-Bo Chen [b,1], Jing-Jing Wang [a], Chao Xu [a], Ming-Liang Zhao [a], Hua-Jiang Dong [c], Hai-Qian Liang [a], Xiao-Hong Li [a], Yue Tu [a], Sai Zhang [a], Chong Chen [a,*], Hong-Tao Sun [a,*]

[a] Tianjin Key Laboratory of Neurotrauma Repair, Pingjin Hospital Brain Center, Logistics University of PAPF, Tianjin 300162, China
[b] Tianjin Key Laboratory of Cardiovascular Remodeling and Target Organ Injury, Pingjin Hospital Heart Center, Logistics University of PAPF, Tianjin 300162, China
[c] State Key Laboratory of Precision Measurement Technology and Instruments, Tianjin University, Tianjin 300072, China

ARTICLE INFO

Article history:
Received 2 March 2017
Received in revised form 2 June 2017
Accepted 3 June 2017
Available online 16 June 2017

Keywords:
Hypothermic-targeted temperature management
Traumatic brain injury
Time window
Effect

ABSTRACT

Although hypothermic-targeted temperature management (HTTM) holds great potential for the treatment of traumatic brain injury (TBI), translation of the efficacy of hypothermia from animal models to TBI patients has no entire consistency. This study aimed to find an ideal time window model in experimental rats which was more in accordance with clinical practice through the delayed HTTM intervention. Sprague-Dawley rats were subjected to unilateral cortical contusion injury and received therapeutic hypothermia at 15 mins, 2 h, 4 h respectively after TBI. The neurological function was evaluated with the modified neurological severity score and Morris water maze test. The brain edema and morphological changes were measured with the water content and H&E staining. Brain sections were immunostained with antibodies against DCX (a neuroblast marker) and GFAP (an astrocyte marker). The apoptosis levels in the ipsilateral hippocampi and cortex were examined with antibodies against the apoptotic proteins Bcl-2, Bax, and cleaved caspase-3 by the immunofluorescence and western blotting. The results indicated that each hypothermia therapy group could improve neurobehavioral and cognitive function, alleviate brain edema and reduce inflammation. Furthermore, we observed that therapeutic hypothermia increased DCX expression, decreased GFAP expression, upregulated Bcl-2 expression and downregulated Bax and cleaved Caspase-3 expression. The above results suggested that HTTM at 2 h or even at 4 h post-injury revealed beneficial brain protection similarly, despite the best effect at 15 min post-injury. These findings may provide relatively ideal time window models, further making the following experimental results more credible and persuasive.

1. Introduction

Traumatic brain injury (TBI) is recognized a worldwide public health issue due to its increased mortality and long-term morbidity (Stocchetti, 2014; Sun et al., 2016). The potential extended therapeutic window of TBI events and the diversity of targets provide opportunities for treatment interventions considering continued speculation of hypothermia's mechanism (Markgraf et al., 2001). Hypothermic-targeted temperature management (HTTM) has been identified as the candidate of neuroprotective treatment in TBI (Nielsen et al., 2013; Polderman and Varon, 2015).

Clinical trials have suggested that the earlier HTTM is initiated, the more likely beneficial effects may be obtained (Clifton et al., 2001), especially preoperatively in focal brain injury which received hematoma evacuation and which had the ischemic/reperfusion pathophysiology (Yokobori and Yokota, 2016). Virtually, many out-of-hospital patients after TBI missed timely hypothermic remedy when arriving at intensive care unit due to multiple factors. Numerous previous basic researches proved consistent efficacy of HTTM affirmatively, whereas clinical findings demonstrated diverse results on account of the heterogeneity of patients in the severity of TBI (Hifumi et al., 2016). Except for the inherent differences between animals and human beings, the highly controlled environment of the animal research laboratory is impossible to replicate in real-life TBI research trials. Moreover,

Abbreviations: TBI, Traumatic brain injury; HTTM, Hypothermic-targeted temperature management; MHT, Mild hypothermia.

* Corresponding authors.
E-mail addresses: chongchen12@hotmail.com (C. Chen), chenmo333@163.com (H.-T. Sun).

[1] Z.W.Y and C.S.B. contributed equally to this study.

http://dx.doi.org/10.1016/j.brainres.2017.06.006
0006-8993/

we found that most laboratorial animals were treated with HTTM immediately or shortly after well establishing of TBI models (Feng et al., 2010; Bramlett and Dietrich, 2012; Jia et al., 2014; Wang et al., 2016), which deviated from the practical therapeutic time of HTTM absolutely.

The overall goal of this study is to find an ideal time window model in experimental rats which was more in accordance with clinical practice when HTTM (target temperatures 32–34 °C) is implemented at different delayed time points after TBI. Our hypothesis is that reasonable time window models of HTTM will make TBI rats experiments more representative rather than controllable. If experimental rats acquire beneficial outcomes by postponed hypothermic therapy, this study can provide more actual theoretical guidance transformed to clinical practice from fundamental research.

2. Experimental procedure

2.1. Animals

Adult male Sprague-Dawley rats (220 ± 10) g were purchased from the Experimental Animal Center of Academy of Military Medical Sciences of the Chinese PAP. All the animals were randomly divided into five groups: the normothermic uninjured group (SHAM group), the TBI with normothermia therapy group (NT group), the TBI with mild hypothermia therapy group initiated 15 min post-injury (MHT15 min group), the TBI with mild hypothermia therapy group initiated 2 h post-injury (MHT2 h group), the TBI with mild hypothermia therapy group initiated 4 h post-injury (MHT4 h group). All animal experiments were carried out in accordance with the US National Institutes of Health Guide for the care and use of laboratory animals.

2.2. TBI model and temperature manipulation

The rats were anesthetized using 2% phenobarbital sodium solution (50 mg/kg body weight) via intraperitoneal injection and surgically prepared for electronic Controlled Cortical Injury (eCCI) (Custom Design and Fabrication, VA, USA) device or sham surgery as previously described (Schober et al., 2016). In brief, the rats were placed in a fixed frame prior to TBI. A circular craniotomy (diameter 4 mm) centered over the right parietal cortex, which was 3.5 mm posterior to the bregma and 2.5 mm lateral to the midline. The skullcap was carefully removed without disrupting the underlying dura and a moderate cortical contusion using eCCI (parameters setting: the amount of deformation 1.6 mm, the piston velocity 4.5 m/s, the dwell time 120 ms) was produced. Sham-operated animals were anesthetized and surgically prepared, but were not induced by cortical contusion.

Rats in the hypothermia therapy group were cooled by an ice blanket machine and rectal temperature probes and temporalis muscle were used to measure brain and body temperatures, respectively. A target rectal temperature of 32-34 °C was achieved at 15 min, 2 h, 4 h post-injury severally and maintained for 4 h; the rats were then rewarmed to normothermia naturally. The rats of sham group and normothermia therapy group were kept at a constant temperature (37 °C) with a heating pad connected to a rectal temperature probe. Throughout the procedure, mean arterial blood pressure was monitored continuously, and pH and heart rate were measured at 30 min before and 2 h and 6 h after TBI.

2.3. Evaluation of the neurological function

The modified neurological severity score (mNSS) was determined 1, 3, and 7 days after brain injury by blinded and trained observers. The composite neuroscore was generated for alterations of motor/sensory functions, reflexes and behaviors so that the maximal score of 18 represents severe neurological deficit while a score of 0 indicates an intact neurological condition. Specifically, the following scores were assessed: (1) raising rats by the tail and walking on the floor (6 scores), (2) sensory tests (2 scores), (3) beam balance tests (6 scores), (4) reflexes absent and abnormal movements (4 scores). One score is awarded for the inability to perform the task or for the lack of a tested reflex.

The Morris water maze test was performed to evaluate learning and memory functions according to the previous described procedure (Radabaugh et al., 2016). The maze consisted of a round black pool (150 cm diameter, 50 cm high) filled with tap water (22-24 °C) to a depth of 24 cm and a black platform 10 cm in diameter and 22 cm in height was placed in the southwest quadrant of the pool. The water was made opaque by black ink for comparison. Briefly, training began one-week prior injury to find a submerged platform hidden 2 cm under the water surface by using a stationary array of cues outside the pool tub. Each rat was tested across four spaced trials starting from four different start positions on post-injury four consecutive days (21–24 days). Then, the platform was removed from the pool and the rats were placed in the pool from the location point most distal to the quadrant 25 d after TBI. Animals movement was recorded using a video tracking system, and the results, including escape latency, platform crossings and percent time in target quadrant were collected and calculated for statistical analysis.

2.4. Brain water content calculations

Water content percentages of brain tissues were evaluated to observe the cerebral edema using the wet-dry weight method (Tado et al., 2014). After anesthesia, the rats were decapitated immediately and two hemispheres were separated after the brains were removed. The wet weight of each hemisphere tissue was obtained 24 h after eCCI, and samples were dried in a constant oven at 75 °C for 48 h and weighed again to obtain dry weight. The percentage of brain water content was calculated using the formula: brain water content (%) = (wet weight–dry weight)/wet weight × 100%.

2.5. Hematoxylin-Eosin (H&E) staining

Brains were dehydrated, embedded in paraffin and then cut into 5 μm thick sections. The paraffin sections were subjected to dewaxing and hydration for H&E staining. The important point is that the slices were differentiated with 1% hydrochloric acid alcohol for 30 s, then counterstained, dehydrated and cleared, respectively. The pathological lesion zones of brains were observed under a light microscope in a blinded fashion.

2.6. Immunofluorescence

The brains of experimental rats were fixed by transcardial perfusion with 0.9% saline and 4% paraformaldehyde (PFA) respectively. The brains were removed and post-fixed overnight in PFA. Coronal brain sections of hippocampal tissues were cut at 25 μm using a vibratome (Leica VT1000S, Germany). The sections were incubated with primary antibodies: rabbit polyclonal antibodies against doublecortin (DCX, 1:100, Abcam: Cambridge, UK), glial fibrillary acidic protein (GFAP, 1:1000, Abcam: Cambridge, UK), Bcl-2 (1:25, Proteintech Group, USA) and Bax (1:25, Proteintech Group, USA) overnight at 4 °C. Oregon Green 488-conjugated (1:200, Invitrogen: Carlsbad, CA, USA) or Alexa Fluor 568-conjugated (1:200, Invitrogen: Carlsbad, CA, USA) secondary antibodies were incubated after reaction with the primary antibodies.