



Changes of inflammatory factors, reactive oxygen species and cognitive function in mice after brain–blast injury

Ying Liu, Yun–En Liu, Chang–Ci Tong, Hong–Xu Jin

Emergency Medicine Department of General Hospital of Northern Theater Command, Laboratory of Rescue Center of Severe Wound and Trauma PLA, Shenyang 110016, China

ARTICLE INFO

Article history:

Received 23 November 2018

Revision 4 December 2018

Accepted 13 December 2018

Available online 21 December 2018

Keywords:

Blast injury

Brain injury

Cognition impairment

Inflammation

Oxidative stress

ABSTRACT

Objective: To study the changes of cognitive function in mouse after brain-blast injury. **Methods:** Forty healthy male C57BL/6 mice were randomly divided into model group and control group. After 24 h of injury, histopathological changes and reactive oxygen species changes were observed under microscope; while changes of inflammatory cytokines content were determined by Western-blot. Four weeks later, Morris water maze method was used to detect the cognition impairment. **Results:** HE staining showed blast induced brain injury in C57BL/6 mice. Compared with normal control group, the expression of IL-1 β , IL-4, IL-6 were significantly increased in brain tissue of model group whereas IL-10 was significantly decreased ($P < 0.05$); ROS expression in the hippocampus of model group mice was significantly increased compared with that in the control group. Morris water maze showed cognition impairment in mice after brain-blast injury. **Conclusions:** Brain-blast injury causes cognition impairment in mice, which may be related to the occur of inflammatory change and oxidative stress in the early stage.

1. Introduction

Brain-blast injury, a kind of blast-related traumatic brain injury (b-TBI), is one of the important causes of brain injury in military activities[1]. The b-TBI patients usually have no obvious external injury, resulting in delayed treatment time and disability finally[2]. Relevant studies have found significant cognitive impairment in the first 2 d after craniocerebral impact injury in rats compared with the

control group[3]. At present, there are few researches on b-TBI in China. Moreover, the change in long-term cognitive function after the injury and the related damage mechanism are far from clear[4,5]. Therefore, we established an experimental animal model of b-TBI in mice to determine the occurrence of brain injury, inflammatory response, oxidative stress and long-term cognitive function changes in mice, which will provide a theoretical basis for improving the treatment efficacy and the living quality of the b-TBI patients.

First author: Ying Liu, Emergency Medicine Department of General Hospital of Northern Theater Command, Laboratory of Rescue Center of Severe Wound and Trauma PLA, Shenyang 110016, China.

E-mail: 752409195@qq.com

Tel: +86 24 28851293

Corresponding author: Hongxu Jin, Emergency Medicine Department of General Hospital of Northern Theater Command, Laboratory of Rescue Center of Severe Wound and Trauma PLA, Shenyang 110016, China.

E-mail: hongxuj@126.com

Tel: +86 24 28851293

Fundation project: This work was supported by Liaoning Provincial Natural Science Foundation of China (No. 201602771).

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2018 Journal of Acute Disease Produced by Wolters Kluwer- Medknow

How to cite this article: Liu Y, Liu YE, Tong CC, Jin HX. Changes of inflammatory factors, reactive oxygen species and cognitive function in mice after brain-blast injury. J Acute Dis 2018; 7(6): 258-261.

2. Materials and methods

2.1. Animal treatments

Forty healthy adult male C57BL/6 mice weighing (23±2) g were purchased from Liaoning Changsheng Biotechnology Co., Ltd. Mice were randomly divided into the control group and the model group. The animals were free to access food and water, and the experiment began after 1 week of adaptive feeding.

2.2. Establishment of models

The blast-injury animal model was made by a preparation device developed independently[6]. The preparation device mainly included air compressor and sensor. Mice were anesthetized and placed on the blast-injury device. The chest and abdomen of mice were protected by protective tubes, and the brain was exposed. The device was turned on and the air was compressed by an air compressor. When the air was compressed to a critical point, the aluminum film would be burst open, and the shock wave generated by the explosion impacted the brain of the mouse.

2.3. HE staining

The brain tissue of each group was retained 24 h after injury, and fixed in 40 g/L paraformaldehyde. The sample was dehydrated with xylene and anhydrous ethanol gradient, then embedded into paraffin. The paraffin samples were sliced. After HE staining, the slice were placed under a microscope to observed brain histopathological changes.

2.4. Western blot assay

Twenty-four hours after brain-blast injury, proteins were extracted from brain tissue of mice, underwent SDS-PAGE electrophoresis and transferred to PVDF membrane. And then the membrane were transferred to 5% skim milk PBST buffer at room temperature for 1 h, and washed in PBST for three times. The appropriate primary antibody of IL-1 β , IL-4, IL-6 and IL-10 (Sigma, USA) were added and incubated overnight at 4 °C. The membrane was rinsed 3 times with PBS, and incubated at room temperature with corresponding secondary antibody (Sigma, USA) for 1 h, respectively. Chemiluminescence was proceeded using ECL Western blotting kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

2.5. Reactive oxygen species (ROS) determination

The mouse brain was cut into 20 μ m thick slices using a frozen microtome. Then, 20 μ L pre-cooled cleaning fluid were added to the entire slice surface. After the cleaning, fluid were carefully removed from the slice, 20 μ L staining solution preheated at room temperature

was added to the slice. Then, the slice was placed in the incubator at 37 °C for 20 min, the stain was carefully removed from the slice, and 20 μ L cleaning fluid were used. The results were observed under fluorescence microscope.

2.6. Morris water maze test

Morris water maze experiment was started 4 weeks after blast injury. The positioning navigation experiment was conducted continuously for 5 d, and the training was conducted twice a day. Keep the water temperature at 25-28 °C, and a fixed position of a quadrant was chosen to put the mice into water. When the mouse found the platform, it was asked to stand on for 10 s. If the mouse could not find the platform within 60 s, it would be gently dragged from the water onto the platform and stopped for 10 s. The space search experiment was carried out the day after the navigation experiment. The second quadrant was selected as the entry quadrant, and the spatial memory ability of the animal was evaluated by the number of platform crossing and time in the target quadrant within 60 s.

2.7. Statistical analysis

SPSS 20.0 software were used to analysis the data. All the data were expressed as the mean \pm standard deviation, and analyzed using the *t*-test. $P < 0.05$ indicated that the difference was statistically significant.

3. Results

3.1. HE staining

HE staining results showed that the structure of brain tissue in the control group was intact and no pathological changes were observed, while the brain tissue of the model group showed obvious vascular dilation, inflammatory cell infiltration, and nuclear retraction and other pathological changes (Figure 1).

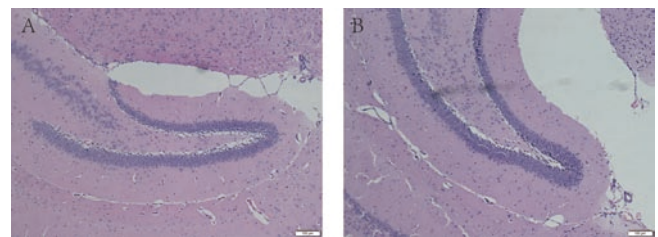


Figure 1. Pathological changes of brain tissue observed by HE staining (HE, $\times 200$).

A. Control group; B. Model group. Scale: 100 μ m.

3.2. Western-blot detection

Compared with the control group, the expression of IL-1 β , IL-4 and IL-6 in the brain of the model group was significantly higher, and the expression of IL-10 was significantly lower (Figure 2).

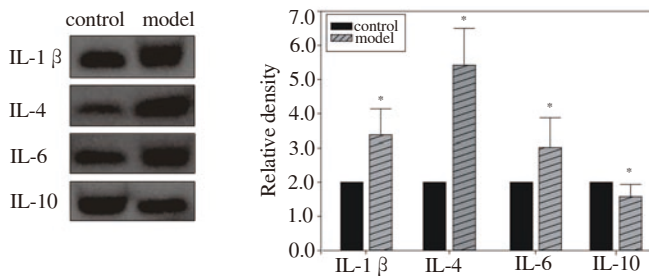


Figure 2. Expression levels of IL-1 β , IL-4, IL-6 and IL-10 of two groups, * $P < 0.05$, compared with control group.

3.3. ROS

After 24 h of the brain injury, ROS expression in the hippocampus of model group mice was significantly increased compared with that in the control group (Figure 3).

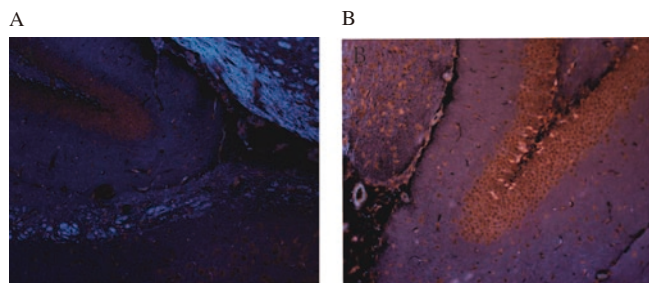


Figure 3. ROS level in brain tissues detected by DHE staining ($\times 200$). A. control group; B. model group.

3.4. Morris water maze test

The average escape latency of the mice decreased over the training days (Table 1). From the 2 d to 5 d, the escape latency of the model group mice was significantly longer than that of the control group mice ($P < 0.05$).

Table 1

Escape latency at different time points in Morris water maze test (Mean \pm SD) ($n=10$).

Group	Training days				
	1	2	3	4	5
Control	56.8 \pm 0.9	44.0 \pm 5.1	38.2 \pm 3.9	33.6 \pm 3.3	29.9 \pm 2.7
Model	57.3 \pm 1.0	58.2 \pm 0.9*	48.5 \pm 5.6*	50.4 \pm 4.1*	41.3 \pm 6.3*

In the day 6, the number of platform crossing was fewer ($P < 0.05$) and time in the target quadrant was shorter ($P < 0.05$) in the model group mice than the control group mice (Figure 4).

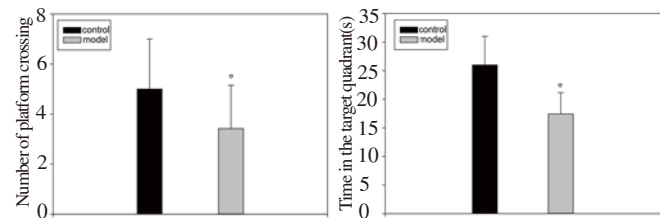


Figure 4. Number of platform crossing and time in target quadrant, * $P < 0.05$ compared with control group.

4. Discussion

b-TBI accounts for a large proportion of explosion related injuries. Blast injury can lead to primary and secondary tissue damage. Secondary injury begins from onset of injury, and lasts a few months or a few years after injury. It can lead to inflammation reaction and the generation of a large number of oxygen radical in the cell, furtherly cause the peroxidation of lipid, protein and nucleic acid, resulting in the injury of nerve cell[7]. The study of b-TBI has become an important research direction of modern military medicine, as well as an important research hotspot of emergency medicine and trauma medicine in peacetime[8]. According to our results, inflammatory factors infiltration and nuclear retraction occurred in brain 24 h after the injury. The expressions of inflammatory cytokines IL-1 β , IL-4 and IL-6 were increased, while the expression of anti-inflammatory cytokines IL-10 was decreased. There were obvious changes in oxidative stress and behavioral memory loss one month after the injury.

In the inflammatory response, pro-inflammatory factors and anti-inflammatory factors are in a state of balance. IL-1 β is a kind of cytokine secreted by activated macrophages[9]. IL-6 is a cytokine produced by monocytes, activated macrophages and endothelial cells, which can induce acute phase protein synthesis, further catalyze and amplify inflammatory reactions and toxic effects, causing tissue and cell damage. IL-6 can also promote intracellular calcium ion flow, and calcium overload causes a series of biochemical reactions in cells, which eventually lead to cell disintegration[10]. IL-10 plays an role in anti-inflammatory process by inhibiting Th1 differentiation, neutrophil activity and NF- κ B activity[11]. The body immediately produces severe stress response after brain blast injury. In pathological conditions, due to the change of cell metabolism, body produces an exceeding amount of ROS. In addition, all kinds of enzymes in the cell also present metabolic disorders, so that ROS can not be metabolized by the body timely. A large number of oxygen free radicals will eventually lead to oxidative stress injury, cell death, excessive activation of apoptosis pathways and irreversible damage[12]. Therefore, long-term cognitive dysfunction after b-TBI in mice may be related to the occurrence of early inflammatory response and oxidative stress.

In conclusion, regulating the expression of inflammatory factors and reducing the oxidative stress response at early stage may be help

to reduce secondary brain injury, improve cognitive function and the long-term quality of life of the patients.

Conflict of interest statement

The authors report no conflict of interest.

Fundation project

This work was supported by Liaoning Provincial Natural Science Foundation of China (No. 201602771).

References

- [1] Bazarian JJ, Cernak I, Noble-Haesslein L, Potolicchio S, Temkin N. Long-term neurologic outcomes after traumatic brain injury. *J Head Trauma Rehabil* 2009; **24**(6): 439-451.
- [2] Liu YE, Tong CC, Zhang YB, Cong PF, Shi XY, Liu Y, et al. Chitosan oligosaccharide ameliorates acute lung injury induced by blast injury through the DDAH1/ADMA pathway. *PLoS ONE* 2018; **13**(2): e0192135.
- [3] Prasada KN, Bond SC. Common biochemical defects linkage between post-traumatic stress disorders, mild traumatic brain injury (TBI) and penetrating TBI. *Brain Res* 2015; **1599**: 103-114.
- [4] Koliatsos VE, Cernak I, Xu L, Song Y, Savonenko A, Crain BJ. A mouse model of blast injury to brain: Initial pathological, neuropathological, and behavioral characterization. *J Neuropathol Exp Neurol* 2011; **70**(5): 399-416.
- [5] Peskind ER, Brody D, Cernak I, McKee A, Ruff RL. Military- and sports-related mild traumatic brain injury: Clinical presentation, management, and long-term consequences. *J Clin Psychiatry* 2013; **74**(2): 180-188.
- [6] Tong C, Liu Y, Zhang Y, Cong P, Shi X, Liu Y, et al. Shock waves increase pulmonary vascular leakage, inflammation, oxidative stress, and apoptosis in a mouse model. *Exp Biol Med(Maywood)* 2018; **243**(11): 934-944.
- [7] Paul Tompkins, Yasvir Tesiram, Megan Lerner, Gonzalez LP, Lightfoot S, Rabb CH, et al. Brain injury: Neuro-inflammation, cognitive deficit, and magnetic resonance imaging in a model of blast induced traumatic brain injury. *J Neurotrauma* 2013; **30**(22): 1888-1897.
- [8] Finnie JW. Neuroinflammation: beneficial and detrimental effects after traumatic brain injury. *Inflammopharmacology* 2013; **21**(4): 309-320.
- [9] Perez-Polo JR, Rea HC, Johnson KM, Parsley MA, Unabia GC, Xu GY, et al. A rodent model of mild traumatic brain blast injury. *J Neurosci Res* 2015; **93**(4): 549-561.
- [10] Elder GA, Gama Sosa MA, De Gasperi R, Stone JR, Dickstein DL, Haghghi F, et al. Vascular and inflammatory factors in the pathophysiology of blast-induced brain injury. *Front Neurol* 2015; **6**: 48.
- [11] Cho HJ, Sajja VS, Vandevord PJ, Lee YW. Blast induces oxidative stress, inflammation, neuronal loss and subsequent short-term memory impairment in rats. *Neuroscience* 2013; **253**: 9-20.
- [12] Kabu S, Jaffer H, Petro M, Dudzinski D, Stewart D, Courtney A, et al. Blast-associated shock waves result in increased brain vascular leakage and elevated ROS levels in a rat model of traumatic brain injury. *PLoS One* 2015; **10**(5): e0127971.