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Alpha tocopherol treatment reduces the expression of Nogo-A and NgR in rat brain after traumatic brain injury

Jinfu Yang, MD, Yongfeng Han, MS, Weiwei Ye, MS, Feng Liu, MD, Kai Zhuang, MS, and Guangyong Wu, MD*

Department of Neurosurgery, Third Xiangya Hospital of Central South University, Changsha, Hunan, China

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ABSTRACT

Background: Neurite outgrowth inhibitor-A (Nogo-A), myelin-associated glycoprotein, and oligodendrocyte myelin glycoprotein are three myelin-associated proteins that act as inhibitors to central nervous system regeneration. Neurite outgrowth inhibitor-A imposes the strongest effect on inhibiting axonal regeneration after traumatic brain injury. Alpha-tocopherol, a member of the vitamin E family, is recognized as an active antioxidative substance. Its use has not been well studied in brain injury research, especially in axonal regeneration research.

Methods: We obtained 99 intact adult male Sprague–Dawley rats (200–250 g) from the Experimental Animal Center of Central South University. We used the modified method of Freeney to generate moderate brain injury in the rats. We injected 600 mg/kg α-tocopherol intraperitoneally daily as traumatic brain injury (TBI) treatment. Then, we performed behavioral tests in the corresponding time point, examined brain tissues after hematoxylin-eosin staining to identify changes in cell morphology, and performed immunohistochemical staining and quantitative real-time polymerase chain reaction to detect the expression of NoGo and Nogo receptor (NgR) in brain tissue.

Results: For the Neurological Severity Scores of rats, there were obvious differences among the three groups at the corresponding time points. Standard hematoxylin-eosin staining showed that the brain structure of a sham-operated group of rats was clear, uniform, and compact. A TBI group exhibited hemorrhage, edema, inflammatory cell infiltration, condensed nuclei, and necrosis. We also saw glial cells and fibrous tissue proliferation. The α-tocopherol–treated TBI group had similar but less severe changes than the TBI group. Expression of Nogo-A and NgR increased after TBI compared with the sham-operated group. However, Nogo-A and NgR expression was significantly lower in the α-tocopherol–treated TBI group compared with the TBI group. Similarly, results showed that functional neurological deficits among rats in the α-tocopherol–treated TBI group were less pronounced than in the TBI group (model group).

Conclusions: Our data demonstrate that α-tocopherol–treated rats had reduced microscopic evidence of brain damage. Alpha-tocopherol reduced Nogo-A and NgR expression in brain tissue after traumatic brain injury and promoted nerve regeneration. Alpha-tocopherol treatment of TBI rats had a neuroprotective role in their recovery.

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* *Corresponding author*. Department of Neurosurgery, First Hospital of Shijiazhuang City, Shijiazhuang, Hebei 050001, China. Tel.: +86 0731 8861 8218; fax: +86 0731 8861 8627.
E-mail address: maoping627@126.com (Y. Han).

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1. Introduction

Although the overall mortality of traumatic brain injury (TBI) has been reduced to 30%, 10% of patients with a mild brain injury are left with a permanent disability. In addition, 66% of moderate and 100% of severe brain injury patients have a permanent disability [1,2]. The high morbidity and mortality is largely attributed to regenerative disorders of the central nervous system. Identification of new medical interventions that promote normal nerve regeneration is a key management issue [2–4]. Barriers to nerve regeneration include lack of neurotrophin, glial scar barriers that develop as part of aberrant healing after TBI, and the absence of controlled axonal growth inhibition [5–7].

Neurite outgrowth inhibitor-A (Nogo-A), myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp) are three kinds of myelin-related protein that are the main inhibitors of central nervous system (CNS) regeneration [8]. Neurite outgrowth inhibitor-A imposes the largest inhibition on axonal regeneration after TBI. Caroni P and Schwab ME succeeded in isolating Nogo-A and cloning the *Nogo* gene [9]. Three expression products were found: Nogo-A, Nogo-B, and Nogo-C. Because Nogo-A exists only in the CNS and possesses strong inhibitory activity on nerve axons, its potential role in healing after TBI has been of great interest [10–13].

Vitamin E is a general term for a group of molecules, including α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienols. In recent years, it has been observed that vitamin E has a characteristic susceptibility to oxidation and is a natural antioxidative. Alpha-tocopherol especially is known as an antioxidative substance [14–18]. A few studies have examined the effect of α-tocopherol on free radical scavenging and axonal regeneration after TBI.

We used a freefall injury animal model to study the effect of α-tocopherol treatment on TBI healing. We examined rat neurological dysfunction after TBI, as well as morphological changes in brain tissue and Nogo-A and Nogo receptor (NgR) expression change.

2. Materials and methods

2.1. Experimental animals

We obtained 99 healthy male Sprague-Dawley rats from the Experimental Animal Center of Central South University. We randomly divided animals into three groups: sham treatment, TBI, and TBI + α-tocopherol treatment. The α-tocopherol (Sigma-Aldrich Corporation, St. Louis, MO) treatment consisted of 600 mg/kg dosing [19] by intraperitoneal injection. Alpha-tocopherol treatment started 6 h after inducement of TBI and recovery from anesthesia. Treatment continued once daily until the animals were killed. The sham group and TBI group received 5 mL saline intraperitoneal injections.

Selected animals were about 2 mo old and weighed 200–250 g. We fed rats a standard diet and gave them free access to water. The testing room was maintained at constant temperature of 23°–25°C and humidity (70%), and the environment was kept quiet. The Ethics Committee of the Central South University approved the study.

2.2. Traumatic brain injury model

We modeled TBIs using the advanced method of Feeney *et al* [20]. We administered 10% chloral hydrate (3 mg/kg) intraperitoneal anesthesia, placed animals in the prone position and used an approximate 2-cm scalp incision to expose the right parietal skull. We made burr holes and expanded the bone window to about 5 mm in diameter. We carefully avoided the sagittal sinus. We placed a sponge under the head and a 4-mm-diameter polyethylene plate in the bone window. Using a fixed vertical guide rod, we hit the polyethylene plate with a 20-g hammer from a 30-cm height. This method induces a moderate brain injury in the right hemisphere. After impact, there were brief limb convulsions and apnea, indicating that an injury had occurred. The sham group underwent only scalp incision and bone window formation, omitting the traumatic injury. The rats were maintained at 37°C during the surgical procedure and after they awoke. After the procedure, we removed nine rats that remained comatose or died.

2.3. Postsurgical evaluation of rat neurological states

Using the Neurological Severity Score (NSS) developed by Schallert *et al* [21] and Chen *et al* [22], we tested movement, sense, balance, and reflex in rats from all three groups. The lowest score was 0, which represents normal neurological function; the highest score was 18. Higher scores represent more severe damage to neurological function (Table 1). In all animals, an investigator who was blinded to the experimental groups performed a battery of behavioral tests at 1, 3, 7, and 14 d.

2.4. Specimen collection and processing

We randomly killed rats 1, 3, 7, 14, and 21 d after TBI. We anesthetized rats with 10% chloral hydrate and made a xiphoid transverse incision along the junction of the diaphragm and thoracic areas, exposing the heart. The apex of the incision was held up to visualize the ascending aorta. We incised the right atrial appendage and rapidly instilled 200 mL saline at 37°C. We next injected a fixative (250 mL, 4% paraformaldehyde in phosphate-buffered saline at 4°C) until the animals were stiff and liver was pale because of a lack of blood. We then harvested brain specimens and placed them in 4°C fixative fluid overnight. Specimens next underwent gradient alcohol dehydration and were paraffin-embedded. Finally, we mounted 5-μm-thick sections on a glass slide for observation.

2.5. Immunohistochemistry analysis

We performed immunohistochemical staining according to the protocol of the Department of Pathology, Third Xiangya Hospital of Central South University, Changsha Hunan, China. Briefly, after proteolytic digestion and peroxidase blocking, we incubated the slides overnight with the primary antibody. All incubations with primary antibodies were carried out

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