Effect of Erythropoietin and Stem Cells on Traumatic Brain Injury

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OBJECTIVE: **To investigate the healing effects of erythropoietin (EPO) and stem cells (SCs) in traumatic brain injury (TBI).**

METHODS: **Twenty-nine Wistar albino rats were used and separated into the following groups: control (C), EPO, SC, and SC + EPO. Group C received a TBI only, with no treatment. In the EPO group, 1000 U/kg EPO was given intraperitoneally at 30 minutes after TBI. In SC group, immediately after formation of TBI, 3 × 10,000 $CD34^+$ stem cells were injected into the affected area. In the SC + EPO group, half an hour after TBI and the injection of stem cells, 1000 U/kg EPO was injected. Before and after injury, trauma coordination performance was measured by the rotarod and inclined plane tests.**

RESULTS: **Seven weeks after trauma, rat brains were examined by radiology and histology. Rotarod performance test did not change remarkably, even after the injury. Compared with group C, the SC + EPO group was found to have significant differences in the inclined plane test results.**

CONCLUSIONS: **Separately given, SCs and EPO have a positive effect on TBI, and our findings suggest that their coadministration is even more powerful.**

INTRODUCTION

Despite many efforts, there are currently no satisfying treatments available for functional impairments after traumatic brain injury (TBI). Fortunately, increasing evidence suggests that stem cell (SC)–based transplantation therapies may be one of the most promising therapeutic strategies for treating functional impairments after TBI,[1] and various types of SCs, including embryonic SCs, neural SCs, and mesenchymal stem cells (MSCs), currently are being investigated for transplantation.[2-4] New experimental strategies focus on neuroregenerative approaches, among which the application of MSCs has gained increasing attention. MSCs, like other SCs, have the capacity of unlimited self-renewal and give rise to differentiated cells from various cell lineages.[5] MSCs, administrated by either direct intracerebral injection or systemic injection after TBI, have been successful in reducing the loss of brain tissue and improving neurologic functional recovery in experimental animal models of central nervous system injury[6-8]; however, SC therapy is hindered by insufficient concentrations of cells within the target tissue and the low survival rate of the cells grafted.[9,10]

Erythropoietin (EPO), essential for erythropoiesis, also is expressed in neurons, astrocytes, and cerebral endothelial cells.[11] EPO is a multifunctional agent with tissue protection–exerting antiapoptotic, anti-inflammatory, antioxidative, angiogenic, and neurotrophic properties.[12,13] EPO shows neuroprotection in animal models of stroke,[14,15] concussive brain injury,[16] and kainate-induced seizure activity.[13] Many researchers have long explored agents with proven in vitro and in vivo neuroprotective effects to assist in repairing damaged nervous tissue.[17]

In the light of previous results, we think that EPO supports SC in the repair of damaged areas. Therefore, we investigated the effects of SC+EPO on motor performance and healing in rats with TBI.

MATERIALS AND METHODS

Rotarod Performance Test

Before the study groups were formed, the rats were put on the rotating shaft for 3 minutes with a speed of 10 cycles/minute and

Key words
- Erythropoietin
- Stem cell
- Traumatic brain injury

Abbreviations and Acronyms
EPO: Erythropoietin
GFAP: Glial fibrillary acidic protein
H&E: Hematoxylin and eosin
MRI: Magnetic resonance imaging
MSC: Mesenchymal stem cell
RECIST: Response Evaluation Criteria in Solid Tumors
SC: Stem cell
TBI: Traumatic brain injury

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then made to walk weekly on the shaft. The average value was recorded as the value of rotarod performance. Each test was performed 3 times for each rat, and the average score was selected.

Bidirectional Inclined Plane Test

The inclined plane was composed of a 28 cm × 30 cm floor covered with a grooved, 1 mm-thick rubber surface that was 20 cm × 30 cm, and 10 cm high on 3 sides. This task evaluated the animal's ability to maintain its body position on a board that was raised incrementally at increasing angles. The rat was placed on the inclined plane with its head down or up and to the right or left. The angle of inclination was then gradually increased towards the vertical position until the rat could no longer remain in place at the starting position. The largest angle at which the rat could maintain a stable position for 5 seconds was recorded. This test was used as an index of animal motor function. Rats were placed on the table upside-down and head-up directions for 5 seconds, the greatest angles values that they could stay were added, and the mean value was obtained. This value was recorded as the rat's bidirectional inclined plane score.

Animals and Surgical Procedures

In this study, 29 Wistar albino 6- to 8-month-old male rats (180 – 250) were used. They were reared under the supervision of a veterinarian, kept in well-ventilated, silent environment, and allowed free access to food and water. The rats were housed in standard laboratory conditions (22 ± 2°C, 50% humidity, 12-hour light/dark cycle) in plastic cages (42 × 26 × 15 cm) that contained 3 or 4 rats each. The study was approved by local ethics committee.

Experimental Dosing

All rats were assigned to 1 of 4 experimental groups: control (C), SC, EPO, or SC+EPO. Before TBI was induced, all animals were familiarized to the rotarod and bidirectional inclined plane test and the scores of rotarod and bidirectional inclined plane test results of the animal were recorded. All the rats were administered a single dose of anesthesia (100 mg/kg ketamine + 5 mg/kg xylazine intraperitoneally). Under deep anesthesia, the rats were placed in prone position on the operating table then secured in a stereotactic frame, and the scalp was incised along the midline. The left side of the skull at the area of the motor cortex was removed (size of craniectomy, 5 mm^2) with a dental drill. The coordinates of the 3 points from the bregma were 3.5 mm rostral/1.2 mm lateral (coordinate A = +3.5, +1.2), 1.7 mm caudal/1.2 mm lateral (coordinate B = −1.7, +1.2), and 3.5 mm rostral/3.7 mm lateral (coordinate C = +3.5, +3.7). From the all groups a 1.5-mm area in depth was resected (Figure 1). Group C received TBI without any treatments. In the EPO group, 1000 U/kg EPO was given intraperitoneally at 30 minutes after TBI. In the SC group, immediately after TBI, 3 ×10,000 CD34 SCs was injected into the affected area. In the SC+EPO group, half an hour after TBI and injection of SC, 1000 U/kg EPO was injected. For all groups, the skull bone removed during surgery was repositioned, and the scalp was sutured. They type of trauma applied was the piece-removing model. The average, equal amount of brain tissue was removed with the help of stereotaxy and a Hamilton needle.

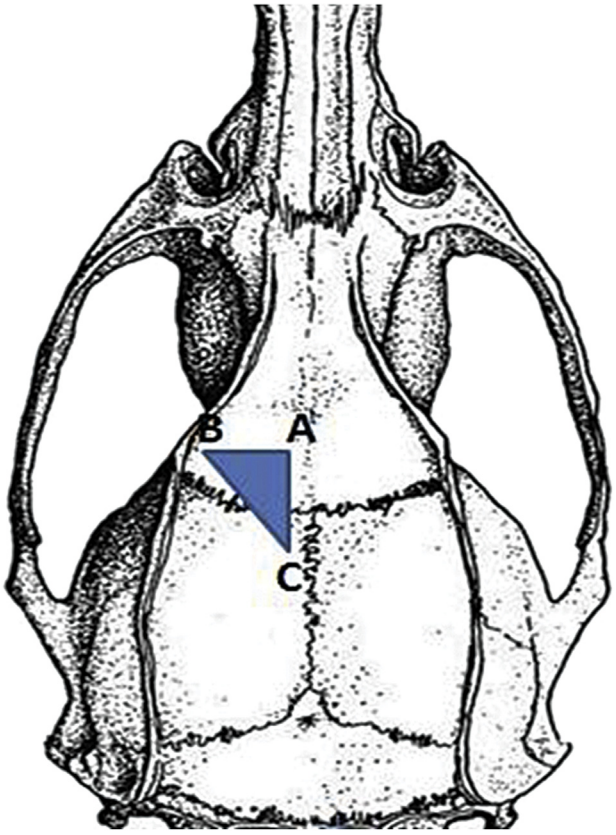

Figure 1. The coordinates of the area in which the damage was created.

Preparation of CD34 SCs

Human SCs were obtained from one pregnant woman, after the woman was instructed to fill out a consent form in which she gave us permission to use her cells in this study. CD34 SCs from cord blood taken were obtained by the use of EasySep Kit (cat. no. 18056, EasySep Human CD34 Selection Cocktail; STEMCELL Technologies, Vancouver, Canada).

Histopathologic Analysis

Brain tissues were immersed in a 10% formaldehyde solution. After fixation, the histologic sections of the brain were cut in the axial plane at 3-μm thickness in a freezing microtome. Histologic sections were stained by hematoxylin-eosin (H&E), Ki-67, CD34, S100, and glial fibrillary acidic protein (GFAP). Then, the preparations were examined under a microscope. The staining ratio of the damaged area, vascularization, and presence of proliferative cells was evaluated in all preparations.

Radiologic Analysis

Animals underwent magnetic resonance imaging (MRI) at 1, 3, 5, and 7 weeks after TBI with a 1.5-Tesla MRI superconducting magnet (Signa Excite HD; GE Healthcare, Milwaukee, Wisconsin, USA) with an 8-channel neurovascular coil. Conventional MRI of cerebral pathologic signal area, the largest axial length was