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Erythropoietin Effects on Pathological Changes of Brain Tissues and Motor Balance Functions after Traumatic Brain Injury in Animal Model

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Abstract

Objectives: In this control trial study we showed the neuroprotective effects of the erythropoietin (EPO) in traumatic brain injury in animal model.
Methods: The research was carried out on 50 male Wistar rats weighing 200 to 250 g. They were divided into two groups of control and case. The rats were anesthetized, right frontal craniotomy was performed and then the brain damage was caused by weight-drop model. In the case group, after 3 hours and again after 24 hours of craniotomy and creation of brain lesions in the right hemisphere, 500 u/kg erythropoietin was injected into peritoneum but in the control group, the rats did not receive any drug. Then the behavior, motor function and balance on the second day and the fourteenth day after injection of erythropoietin were evaluated. After that, the rats were killed and the brain tissues sent to the laboratory for pathological assessment of brain tissues.
Results: The average of cross-sectional damage in the case group that received erythropoietin drug was reported 22.55% and in the control group 37.41%, and the motor balance function after fourteenth day in the group that received erythropoietin was 69.12% and better in comparison with the control group (46.27%) that did not receive any drug.
Conclusions: erythropoietin has a protective effect on neurons and improves the sub-acute changes in head after brain injury and increases the motor balance abilities in rats.

Keywords: Traumatic Brain Injuries, Erythropoietin, Neuroprotection

1. Background

Accidents are the third cause of death in human populations (1). Traumatic brain injury is the major cause of morbidity and mortality in young adults (2). Annually, about 5 million people around the world die by accidents and the majority of them suffer from complications and neurological disability due to the traumatic brain injury (3). Traumatic brain lesions are the first cause of neurological disability in young adults. Thus, many studies have been conducted in the field of assessment of traumatic brain lesions, diagnostic measures, medical and surgical treatments and rehabilitation; and the investigations in this field have been continued due to the high level of mortality and morbidity (4).

Generally, traumatic lesions of the brain tissue are divided into two categories: primary damage and secondary damage. The primary damage is developed at the moment of injury but the secondary damage is developed when the initial injury starts and it can continue for months or even years (5). Treatment in the field of primary damage of brain parenchyma is very limited because neurological cells are non-proliferated but all the attention is focused on follow-up medical care, treatment and preventing the development of the brain's secondary damage.

Secondary damage refers to the development of brain cell damage when the primary damage occurs in the areas that are less damaged or undamaged, and it can continue after a month or years. In the secondary damage of brain, a cascade of biochemical and physiological changes occurs and causes more damages to the neurons in the affected area. The secondary damage causes the primary injury to spread and worsen the patients' condition and also

worsen the complications after trauma. The secondary damages are really important for neurosurgeons because many therapies and interventions have been focused on them (6).

The basic mechanism of secondary damage development is the disruption of calcium homeostasis in brain. Due to the trauma, extra calcium is entered into the cells through the membrane channels and membrane receptors. Also, trauma causes depolarization of cell membrane and as a result, ca + 2 enters into the cell through voltage-sensitive channels. Traumatic brain injury causes increase in the membrane potential leading to the excitatory release of amino acids through the Na + / k + pump in the membrane. Theses excitatory amino acids like glutamate, activate some receptors like N-methyl- d- aspartate (NMDA) that are a gateway for Na+ and Ca+ entrance to the cells. Stimulatory neurotransmitters cause cell damage by several mechanisms: acute increase in Na+ and Ca entrance into the cells and then cell swelling, increase in Ca+ entrance into the cells, exit of K+ from the cells, activation of protolithic enzymes, production of oxygen radicals and synthesis of nitric oxide (7).

Erythropoietin is a glycoprotein that is produced by the kidneys. It is well known that Erythropoietin stimulates production of red blood cells in bone marrow. Protective effect of Erythropoietin has been proven in ischemic lesions of the brain and spinal cord. The effect of this drug on traumatic brain lesions has been proven (8). Assessments have showed the strong protective effects of erythropoietin on spinal cord against ischemia and mechanical trauma in rats (9, 10). Erythropoietin has protective effects by several ways: decreasing apoptosis cells, disabling of inflammatory cytokines, activating endothelial progenitor cells, stimulating angiogenesis and decreasing lipids peroxidation (11-13). There are several studies carried out to show the effect of erythropoietin on recovering the spinal cord and brain of traumatic models in rats. To promote previous studies, in this study we evaluate the effect of erythropoietin on the behavior, balance control and histopathological changes in traumatic brain injury rats.

2. Methods

2.1. Subjects

This study was a clinical trial and was done on 50 male Wistar rats weighing 200 to 250 g. The rats were divided randomly into two groups of 25. In the case group, after 3 hours and again after 24 hours of craniotomy and creation of brain lesions in the right hemisphere, we injected 500 u/kg erythropoietin into peritoneum. In the control group, craniotomy and brain lesions in the right hemisphere were done on the rats but in this group, the rats did not receive any drug (include erythropoietin) after creation of traumatic brain injury.

2.2. Methods

First, the rats were anesthetized, right frontal craniotomy was done for them and then the brain damage was caused by weight-drop. After 14 days, the brain was removed and samples were sent to the pathology laboratory to assess the pathological findings and the extent of damage.

In our study, we anesthetized the rats by two drugs, ketamine 10% and midazolam. Ketamine and midazolam each were injected in intra-peritoneal at a dose of 50 to 100 mg/kg and 5 mg/kg. The rats were usually anesthetized 5 minutes after injection and they were prepared for the experiment.

Considering the previous literatures, we used the weight-drop model for traumatic brain injury. A bar weighting a 12 g counter-weight was used for this purpose. This bar had 10 cm length and 5 mm diameter. It was released towards the Dura mater without any uncoated bone and without any additional force through a tube 15 cm from a distance of 8 cm on the rats' brains. This was done several times for all the samples. Craniotomy in rats was performed in RAZI vaccine and serum research institute and it took 2 months.

After the surgery, the rats were placed into their cases and warmed with a warmer; and they became conscious 2 or 3 hours later. After recovery, most of the rats had left hemiparesis and the weakness of their upper limb was more than their lower limb.

Some of the rats died during induction of anesthesia due to the respiratory and cardiac arrest and some of them were not conscious after the surgery. The rats that died around the time of surgery were excluded from this study.

To evaluate the effect of erythropoietin on traumatic brain injury in rats, two ways were considered: the first was the evaluation of behavior, motor function and balance on the second day and the fourteenth day after injection of erythropoietin and the second was the pathological findings in the laboratory.

There are many methods for evaluation of the motor function in rats; for examples, the pole test, beam walking test, wire hang test, and footprint test; but in this study, we used angle board test to evaluate the motor function and behavioral experiments in order to investigate the effect of erythropoietin. For this purpose, the rats were trained before the test. Usually, 3 to 4 times each day, the rats were placed on the inclined board devices to learn how to exercise three days before surgery, or they were excluded from

the study if they did not have motivation to do the test. After training, the rats were prepared for surgery.

The first evaluation on the second day was done with an inclined board in order to assess balance and motor function. Plates were inclined at 45 degrees and rats were placed on them. The period during which the rats could maintain their balance on an inclined board were calculated and considered as the data. After fourteen days, motor function and balance were evaluated again, and finally data were analyzed.

On the fourteenth day, after the motor functional and behavioral tests were performed, the rats were killed by injection of 3 cc KCL solution in the intra-peritoneal; rat's brain were immediately isolated and the samples such as brain, cerebellum and primary parts of the spinal cord were fixed in formalin and 24 hours later, they were sent to the pathology laboratory. In the pathology department, the brain samples were cut in to the slices of 1.5 to 2 mm thickness and were placed on slides for microscopic evaluation. Damage to the cross-sectional area was calculated as a percentage of the total brain.

2.3. Statistics

Data were collected and analyzed with SPSS. Independent T-test was used to analyze the data, and P value < 0.05 was considered to be statistically significant. In addition, the data were compared and shown by

3. Results

According to the results, erythropoietin drug reduced the cross-sectional area injuries of brain in rats. The average of cross-sectional damage in the case group that received erythropoietin drug was 22.55% and in the control group 37.41%; therefor, a significant difference was reported in cross-sectional damages between the two groups (P value < 0.001) (Figure 1).

No significant difference was reported in evaluation of motor function on the second day after brain damage between the case group that received erythropoietin drug and the control group (P value: 0.4) (Figure 2).

Evaluation of motor function on the fourteenth day after the brain damage indicated a significant difference in the final status of motor function between the case group that received erythropoietin drug and the control group (P value = 0.03) (Figure 3).

Evaluation of motor function and balance on the second day after the brain damage showed no significant difference between the rats that received erythropoietin drug and were tested with an inclined board and the control group (P value = 0.2) (Figure 4).

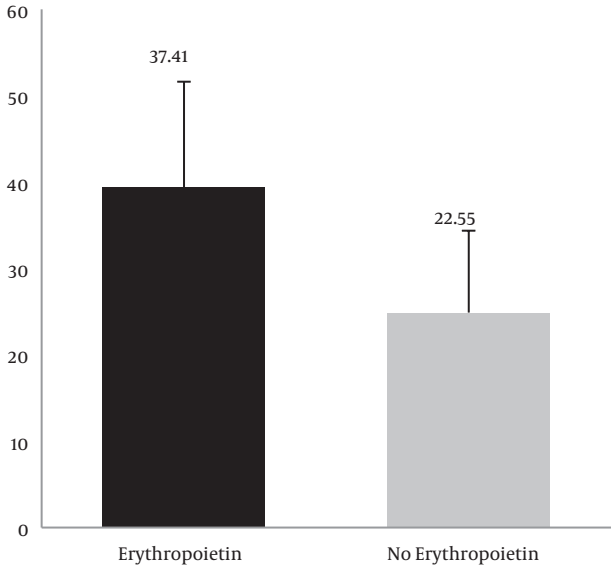

Figure 1. Cross-Sectional Damage

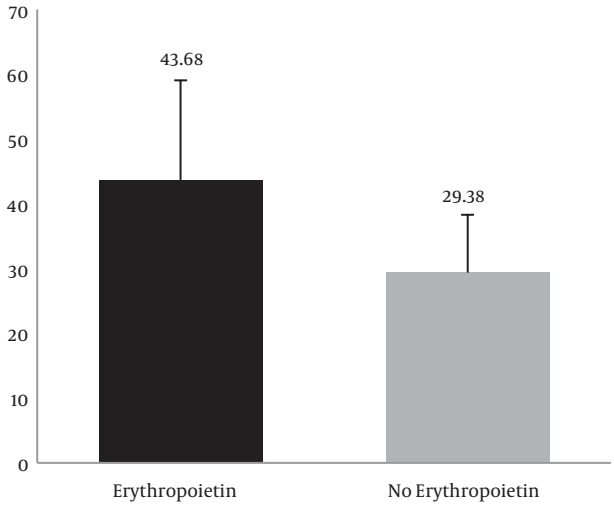

Figure 2. Brain Damage

Evaluation of motor function and balance on the fourteenth day after the brain damage and inclined board test indicated that time balancing on the board was more in rats that received erythropoietin drug in comparison with the control group (P value = 0.01) (Figure 5).

4. Discussion

The best results obtained from this study are related to the pathology results. The pathological changes in brain

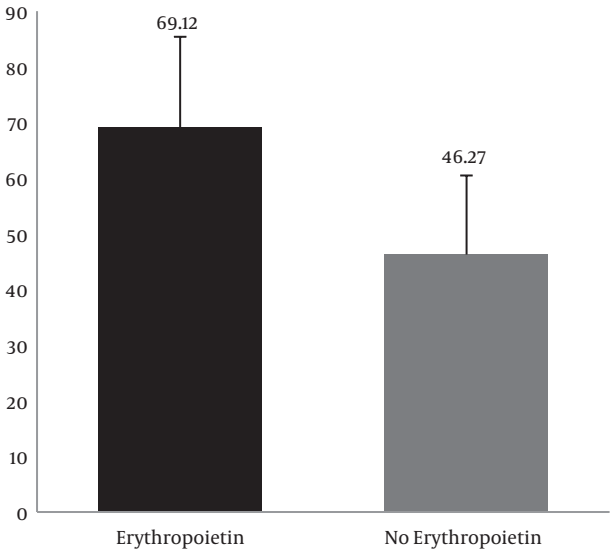

Figure 3. The Final Status

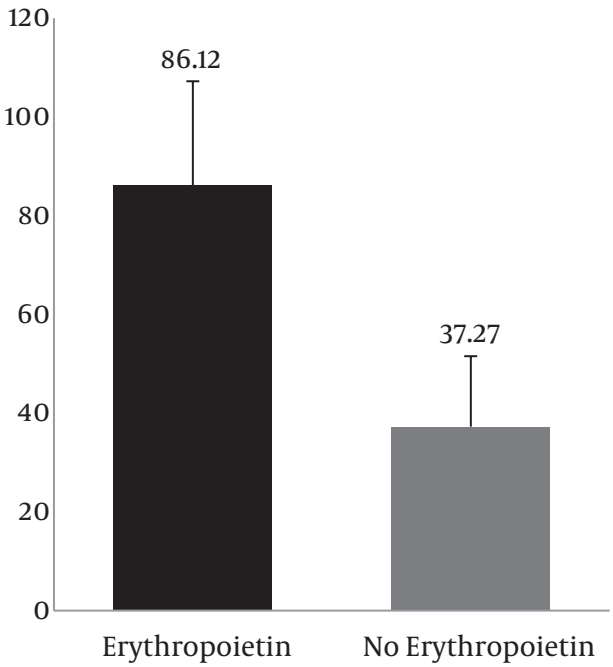

Figure 4. Motor Function

tissues in the case group that received erythropoietin included: increased neovascularization, reduction in the extent of contusion, angiogenesis with thin-walled vessels and sedimentation of hemosiderin (Figure 6). Pathological changes in the brain tissues in the control group that did not receive Erythropoietin included: Increased infiltration of inflammatory cells including lymphocytes and plasma cells and histiocytes much less, formation of fibrous tissue, necrosis, abundant neutrophils, angiogenesis with thick-walled vessels, proliferation of endothelial cells, extent of high contusion and hemorrhage (Figure 7).

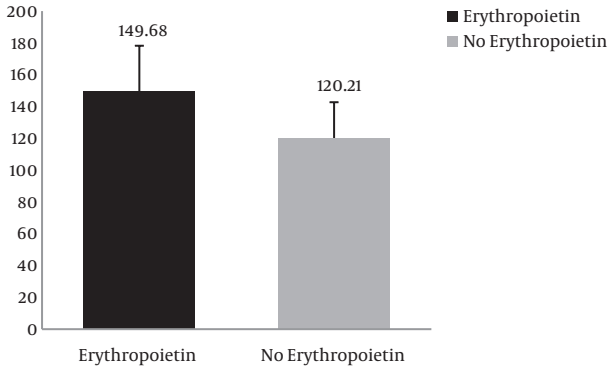

Figure 5. Fourteenth Day

In our research, the microscopic assessments after two weeks demonstrated sub-acute changes in in head after brain injury, and it was clear that erythropoietin drug has good effects in sub-acute phase on the traumatic brain injuries. As the novelty of our research, we found that erythropoietin can improve the motor balance abilities of rats after treatment with erythropoietin.

There are many literatures showing the effect of erythropoietin on improvement of the brain and spinal injuries and its neuroprotective effect in-vitro (14, 15) and in vivo with stroke model (16, 17). In contrast to that literature, in our study we injected erythropoietin into the peritoneum 3 hours and again 24 hours after development of brain injury and also we investigated microscopic evaluation of brain with motor balance skills.

According to the studies conducted by scientists, the hypoxia and CNS injuries can increase the level of erythropoietin hormone and the number of receptors of erythropoietin in CNS (18). Recent studies in animal model demonstrated that erythropoietin is an effective hormone that increases the improvement of neurological disorders after spinal cord injuries (19).

Research articles about this topic in recent years have demonstrated the effect of erythropoietin on the protection of neurons after traumatic brain injuries. Also, in some studies erythropoietin is a valuable hormone in protection of brain in neonatal brain injuries and other neurological disorders such as epilepsy (20, 21).

Some of the studies in animal models showed that recombinant human erythropoietin can decrease the exten-

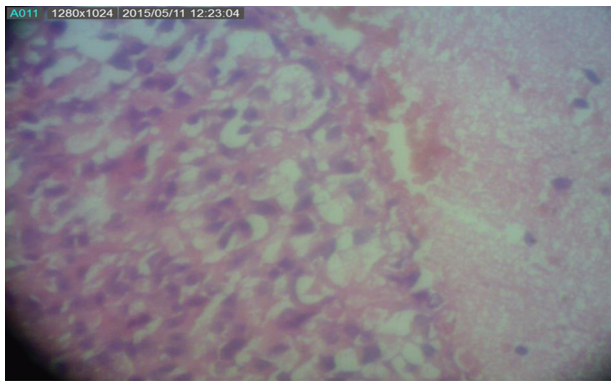

Figure 6. Pathological Assessments of Brain Tissues Demonstrated the Increase of Neovascularization, Increase of Inflammatory Macrophages in Trace Amounts in Rats that Received Erythropoietin After Brain Lesion.

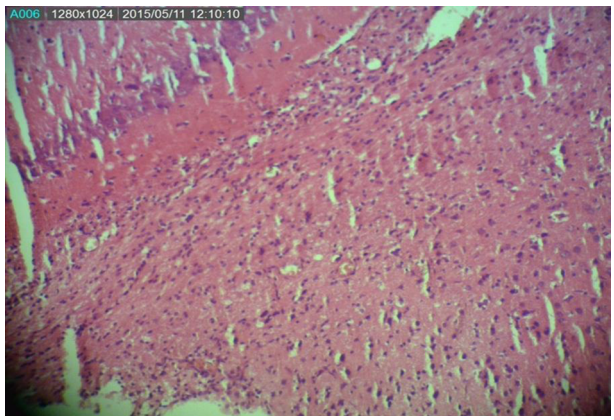

Figure 7. Increasing Infiltration of Inflammatory Cells Including Lymphocytes and Plasma Cells and Histiocytes with Formation of Fibrous Tissue, and Necrosis in Pathological Finding of Brain Tissues in the Control Group that Did not Received Erythropoietin Drug.

sion of post-traumatic brain edema in traumatic brain injury (TBI) (22).

In spite of of several studies in neuroprotective effect of erythropoietin on animal model, there are limited studies on neuroprotective consequences of erythropoietin in human and contradictory consequences in human (23). The clinical trials have only been conducted since 2011, and most of the studies on human have not yet finalized (24). Therefore, further studies are needed in this regard and some other unpublished documents may help to explain the protective effects of EPO on TBI.

4.1. Conclusions

According to our research and other similar papers, EPO has a protective effect on neurons and it can improve the sub-acute changes in head after brain injury and increase the motor balance abilities in rats. Further studies are needed to investigate the effect of EPO on human brain injuries.

Acknowledgments

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Footnote

Conflict of Interest: None.

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