# Research and application of the Recombinant human erythropoietin about neuroprotection of Patients with craniocerebral injury

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**Abstract: Objective;** To explore the effect of recombinant human erythropoietin(rhEPO) for the neuroprotection in patients with craniocerebral injury and its clinical application. **Methods;** The 80 cases selected that were from ICU patients with craniocerebral injury, the 50 cases chose from 80 cases as the standard data were divided randomly into rhEPO group with 30 cases and control group with 20 cases. The rhEPO group was hospitalized and treated，besides conventional treatment，with 10000 IU/time of the rhEPO by the subcutaneous injection in the 3rd, sixth, ninth and 12th day. The control group was given with the conventional treatment only. All the patients were assessed with neuroglobin, hemoglobin, c-reactive protein values, APACHE Ⅱ scores, GCS scores and their ventilation time on the day in hospital and the fourth day, seventh day, tenth day, 14th day after they had been in the hospital. **Results;** The neuroglobin in the rhEPO group is higher than the experimental group that was with statistical difference ($F_{group}$=9.979，P<0.05；$F_{time}$=11.56，P<0.01)；The hemoglobin in rhEPO group is higher than the control group that was with statistical difference ($F_{group}$=20.26，P<0.01；$F_{time}$=22.34，P<0.01);The APACHE Ⅱ score in the rhEPO group is lower than the control group that was with statistical difference ($F_{group}$=9.339，P<0.05；$F_{time}$=13.749，P<0.01)；The ventilation time in the rhEPO group is shorter than the control group that was with statistical difference($P$<0.05).GCS score increased with treatment, but there is no significant difference between the two groups ($F_{group}$ =2.679, P>0.05; $F_{time}$=3.796,P<0.05).Before and after treating, C reactive protein expression in patients of the rhEPO group and the control group has no significant change, no statistical difference ($F_{group}$=0.431，P>0.05；$F_{time}$=1.123，P>0.05). **Conclusions**; in the patients with craniocerebral injury, the rhEPO using could promote the expression of neuroglobin, promote the expression of hemoglobin, and meanwhile could make APACHE Ⅱ score decreased and shorten ventilation time, but there were no the effects on the expression of CRP and GCS scores.

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**Key words:** RhEPO, Craniocerebral Injury, Neuroglobin, Hemoglobin, C Reaction Protein, APACHE Ⅱ, GCS, Ventilation Time.

## Introduction

With the development of national economy and transportation, the incidence of craniocerebral injury and the rate of disability to death increased year by year, neuroprotection has become the research hotspot now. Erythropoietin (EPO) is a hormone-like substances secreted by the kidney and liver.It is widely used in the treatment of anemia due to a variety of causes in recent years. Studies have found that EPO receptor (EPOR) exist widely in all kinds of non-hematopoietic tissue [1] which has a variety of non hematopoietic function but is a kind of systemic protective cytokine [2].The experiment has proved that the central nervous system also can produce EPO and EPO receptor which has a variety function of neural protection [3], but its mechanism is unclear. Neuroglobin（NGB) was first observed in 2000 by Germany Burmester. It is mainly expressed in the brain tissue and is named as the third type of globulin to carry oxygen [4,5].NGB can combine with oxygen reversibly and has a high affinity for oxygen and is able to deliver oxygen to brain tissue specificity which proving its neuroprotection effect.

Existing animal experiments [6] have showed that the expression of NGB in traumatic brain injury rats can be induced by Recombinant Erythropoietin (Recombinant Human Erythropoietin, rhEPO). But the research about clinical application of rhEPO affecting patients with craniocerebral injury brain is rare. In our study, through the clinical application of rhEPO, We observe its effects on neuroglobin expression in craniocerebral injury patients, at the same time monitoring in patients with hemoglobin, c-reactive protein (C reactive protein, CRP) expression and Glasgow coma scale (GCS), Acute Physiology And Chronic Health Evaluation system Ⅱ (Acute Physiology And Chronic Health Evaluation Ⅱ, APACHE Ⅱ score), monitoring patients with mechanical ventilation time and investigating the neuroprotection effect and mechanism of rhEPO.

## 1. Materials and Methods

**1.1. Case selection** Collected 80 cases of craniocerebral injury patients in ICU of the first affiliated hospital of Zhengzhou University from August 2012 to June 2013.They were diagnosed by CT, conformed to the inclusion criteria:1)The patients were send to the hospital within 24 hours after trauma; 2) The GCS scores are range from 3 to 12;3)The patients has no blood coagulation dysfunction.Exclusion criteria:1)There are organic mental disorder or other nervous system diseases in the patients;2)Polycythemia;3)With the use of erythropoietin contraindications to patients.Weeding out criteria:1)The time living in ICU is less than 2 weeks;2) Patients which have adverse reactions after the use of erythropoietin;3)HCT rising over time, male > 0.50 L/L (normal 0.40 to 0.50 L/L) normal, female > 0.45 L/L (normal 0.37 to 0.45 L/L).At last,50 cases choosing from 80 cases conforming to the standard data were divided randomly into rhEPO group with 30 cases and control group with 20 cases. The age, gender, GCS score, damage type etc in two groups have no statistically significant difference (P > 0.05). This study has been passed by the ethics committee review and has obtainned consent from the patient or family.

**1.2. Therapeutic Method:** The control group was given with the conventional treatment including dehydration of intracraninal pressure, trophic nerve and local mild hypothermia therapy, etc. The rhEPO group was treated, besides conventional treatment， with 10000 IU/time of the rhEPO by the subcutaneous injection in the 3th, 6th, 9th and 12th day.

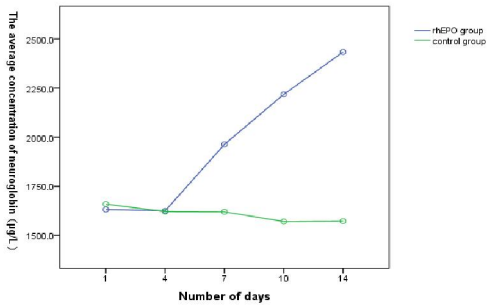
**1.3 Obvervational Index:** Monitoring data on the day in hospital, the hospital 4 days, 7 days, 10 days, 14 days: ①General indicators: records of the patients' vital signs, including temperature, breathing rate, heart rate, blood pressure, the GCS scores and APACHE Ⅱ scores. At the same time monitor patients with mechanical ventilation time.②Hemoglobin,CRP detection: venous blood was collected 5 ml and sending it to the first affiliated hospital of zhengzhou university biochemistry room to inspect within 2 hours, recording the results.③Neuroglobin detection: Venous blood was collected 3 ml, using the anticoagulant sodium citrate anticoagulation, centrifugal in serum within 2 hours, place - 70 ℃. Late unified thaw, using the method of enzyme-linked immunosorbent (Enzyme - linked immune- sorbent assay, ELISA) detect the concentration of neuroglobin in serum.

**1.4. Statistical Processing:** Use SPSS 19. 0 statistical software for statistical analyse. The expression of measurement data is to mean + / - standard deviation. Statistical methods use analysis of variance of repeated measurement data.

## 2. Results

**2.1.** The comparison of concentration of neuroglobin between rhEPO group and control group: The neuroglobin in the rhEPO group is higher than the control group(see table 1), and with the time changing, the expression of neuroglobin in the rhEPO group of patients increase highly. (Figure 1)

Figure1, the average concentration of neuroglobin changing over time in two group




**2.2.** The comparison of concentration of hemoglobin between rhEPO group and control group: The hemoglobin in the rhEPO group is higher than the control group,and with the time changing, the hemoglobin expression in both groups were increased, but the amplitude in the control group is small. (see figure 2).

Figure2, the average concentration of hemoglobin changing over time in two group

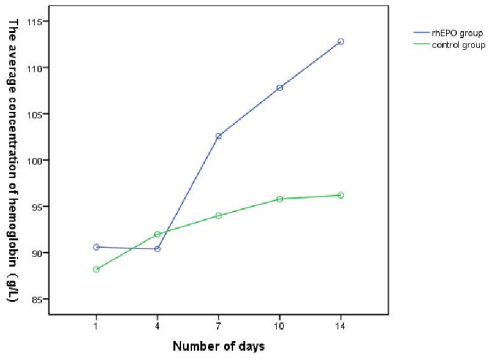
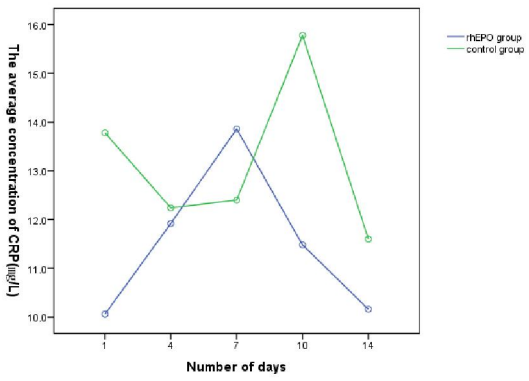



Figure3, the average concentration of CRP changing over time in two group



**2.3.** The comparison of concentration of CRP between rhEPO group and control group:There is no statistical differences between the two groups (Table 3)

**2.4.** The comparison of scores of APACHE Ⅱ between rhEPO group and control group:The score of APACHE Ⅱ in the rhEPO group is lower than the experimental group(see table 4), and with the time changing, the scores of APACHE Ⅱ in both groups were decreased, but the amplitude in the control group is small.

**Table 1 Levels of neuroglobin in rhEPO group and control group at different times**

| | n | 1st day | 4th day | 7th day | 10th day | 14th day |
|---|---|---|---|---|---|---|
| RhEPO Group | 30 | 1631.8± 179.0 | 1625.2± 228.4 | 1963.4± 190.0 | 2218.0± 237.5 | 2733.2± 420.9 |
| Control Group | 20 | 1659.2± 72.7 | 1621.4± 126.6 | 1620.2± 190.9 | 1570.8± 268.2 | 1572.6± 180.4 |

$F_{group}$=9.98，$P$<0.05；$F_{time}$=11.56，$P$<0.01

**Table 2 Levels of hemoglobin in rhEPO group and control group at different times**

| | n | 1st day | 4th day | 7th day | 10th day | 14th day |
|---|---|---|---|---|---|---|
| RhEPO Group | 30 | 90.6±4.7 | 90.4±5.6 | 102.6±5.6 | 112.4±4.3 | 119.0±3.8 |
| Control Group | 20 | 88.2±6.0 | 92.0±4.8 | 94.0±4.2 | 95.8±3.8 | 96.2±5.3 |

$F_{group}$=20.26，$P$<0.01；$F_{time}$=22.34，$P$<0.01

**Table 3 Levels of CRP in rhEPO group and control group at different times**

| | n | 1st day | 4th day | 7th day | 10th day | 14th day |
|---|---|---|---|---|---|---|
| RhEPO Group | 30 | 10.1±3.0 | 11.9±3.0 | 13.9±5.1 | 11.5±4.5 | 10.2±2.7 |
| Control Group | 20 | 13.8±5.8 | 12.2±5.0 | 12.4±6.0 | 15.8±6.8 | 11.6±5.7 |

$F_{group}$=0.43，$P$>0.05；$F_{time}$=1.12，$P$>0.05

**Table 4 Levels of APACHE Ⅱ in rhEPO group and control group at different times**

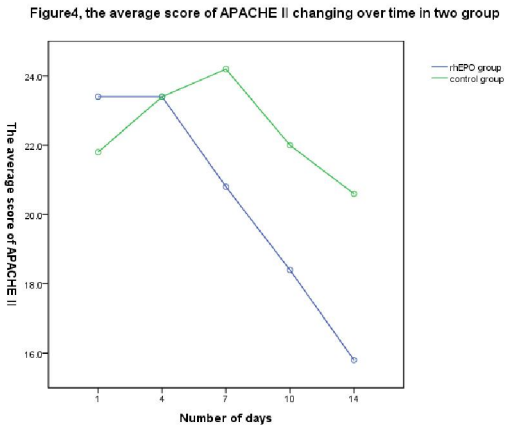
| | n | 1st day | 4th day | 7th day | 10th day | 14th day |
|---|---|---|---|---|---|---|
| RhEPO Group | 30 | 23.4±2.5 | 23.4±2.4 | 20.8±1.5 | 18.4±0.5 | 15.8±1.1 |
| Control Group | 20 | 21.8±3.2 | 23.4±2.6 | 24.2±9.2 | 22.0±0.7 | 20.6±0.6 |

$F_{group}$=9.34，$P$<0.05；$F_{time}$=13.75，$P$<0.01

**Table 5 Levels of GCS in rhEPO group and control group at different times**

| | n | 1st day | 4th day | 7th day | 10th day | 14th day |
|---|---|---|---|---|---|---|
| RhEPO Group | 30 | 6.0±1.6 | 7.0±1.2 | 7.6±0.6 | 9.2±1.1 | 10.8±0.8 |
| Control Group | 20 | 6.6±1.5 | 8.2±1.3 | 8.4±1.3 | 10.0±1.2 | 11.6±0.6 |

$F_{group}$=2.52，$P$>0.05；$F_{time}$=42.33，$P$<0.01




**2.5.** The comparison of scores of GCS between rhEPO group and control group: The GCS score increased with treatment, but no statistical difference between two groups (see table 5)

**2.6.** The comparison of ventilation time between rhEPO group and control group: the ventilation time of rhEPO group is shorter than the control group.(see table 5)

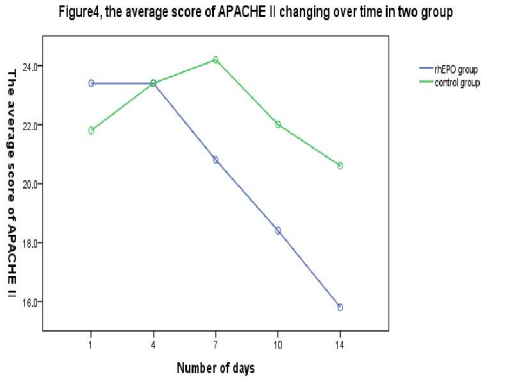



**Table 6 comparison of ventilation time between the two groups**

| | n | Ventilation Time |
|---|---|---|
| RhEPO Group | 30 | 175.0±11.2 |
| Ccontrol Group | 20 | 205.8±9.7 |

*P*=0.002(*P* <0.05), t=-4.662

## 3. Discussion

With the rapid development of social culture, economy and traffic, the incidence of craniocerebral injury in the general population increased year by year, craniocerebral injury patients with poor prognosis, slow recovery of nervous system, brings a heavy burden to the family and the society.How to reduce the harmful effects of craniocerebral injury, strengthen the neuroprotection, has become the research hot spot.

There is not a perfect neuroprotection measures at present. It is well known that the key treatment of patient recovery after craniocerebral injury is the neuroprotection. In addition to the primary brain injury, craniocerebral injury also include a lack of secondary brain injury,like ischemia and brain swelling and so on. The main advances in the recent research about craniocerebral injury is in the secondary damage of neurons,the aim of variety of treatments is to alleviate the secondary neurons injury, to realize the neuroprotection effect, improving the prognosis of patients.Traditional nerve treatment includes continuous physical cooling head, hyperbaric oxygen therapy and so on. In recent years,the discovery and application of neuroprotection agent has become neurobiological research hotspots and difficulties. It has attracted more and more attention. Nerve protectant realize the role of protecting nerve cells by scavenging free radicals, stabling of cell membrane, inhibiting peroxide and so on [7].At present, it was found and applied variety of neural protectants,mainly including: calcium channel blockers, calcium channel regulator, glutamate release inhibitors, GABA receptor agonist, free radical scavenger, intercellular adhesion factor antibody, nerve protection and nutrition drugs, etc. From many research reports, most of the nerve protectant have obvious effects in animal experiments,but in the clinical application its effect is poor;And also because of serious drug side effects, some medicines is limited in the clinical application.Therefore, looking for a new type of neural protectants or application nerve protectant combined of different kinds has become a nerve protectant research focus at present.

EPO is a cytokines produced by embryo liver and kidney which can promote erythropoiesis.Existing experiments have proved that EPO is widely expressed in the brain tissue of rodents and human including new cortex, hippocampus, yan leaves, the amygdala, internal capsule, midbrain, neurons, astrocytes, oligodendrocytes, and endothelial cells[8-9].EPO is an essential growth factor for normal nervous system development. EPO receptor are widely expressed in central nervous system, including neurons and glial cell nucleus endothelial which has neuroprotection effect[10].In our study, it is confirmed that patients with craniocerebral injury patients after using of rhEPO has higher neuroglobin levels and higher hemoglobin levels, APACHE Ⅱ score decreased, shortening the time of patients with mechanical ventilation, explaining that rhEPO could have neuroprotection effect. At the same time,the concentration of CRP in experimental group and control group in patients has no statistical difference, instructing that rhRPO for patients did not cause obvious inflammation, instructing that drug has little side effects. In a variety of experimental animal models, the neuroprotection effect of EPO has been confirmed, including the craniocerebral trauma, stroke,

spinal cord injuries, alzheimer's disease etc.[11]On the basis of the theory of previous animal experiments, we directly conducted clinical trials of rhEPO under the condition of informed consent of the patient's family members and patients, giving the neuroprotection of rhEPO more intuitive display.

In recent years, studies have found that [5] rhEPO has a nerve protective effect on cerebral ischemia.Our experiment explore the neuroprotection effect of rhEPO by exogenous application.

In this experiment, after the use of rhEPO, the concentration of NGB increased.NGB is a newly discovered type of globulin which can carry oxygen [4, 5], because of the kin relationship of NGB and myoglobin, considering that NGB has the oxygen transfer function [12].NGB'S DNA structure is the classic "three - over - there" screw sandwich folded structure, and its basic structure is a structure of hemoglobin and a protein hemoglobin peptide chain. Experiments have confirmed that neuroglobin has iron porphyrin as the same as hemoglobin and myoglobin.But in protein structure and genetic make-up, the difference is bigger. Neuroglobin is unique in brain evolution and function[13]. This is also its basis structure of oxygen transfer function. Studies have shown that hypoxia can stimulate the expression of NGB mRNA [14,15].NGB combined with oxygen reversibly, and has a high affinity for oxygen, can deliver oxygen to brain tissue specifically,improving brain hypoxia, realizing the effect of neuroprotection. Besides of transporting oxygen, HGB and MGB also have the role of clearing nitric oxide (NO). And NGB has the similar structure with them, and high expression of NGB has been discovered in the normal adult liver tissue and embryonic kidney, and there's a lot of NO in these organs metabolic process, seeming to have showed that NGB has the function to clear the NO similar as HGB, MGB, participate in the metabolism of NO, thus reducing the NO damage to the nervous system [16].

How to induce increasing the expression of NGB, and make it play a role to protect the nervous system is of great significance. In a research[6] discussing how rhEPO influence the expression of NGB after injury rats cortex and exploring its significance in brain damage,we found that surrounding area of combatting brain tissue slice which has immunohistochemical examination in rhEPO intervention group expressed obviously higher NGB than that of pure craniocerebral injury group, showing that rhEPO injection can improve the expression of NGB in the rats of traumatic brain injury. The specific mechanism of how RhEPO induce expression of NGB raised is not fully clear. There are also studies have shown that EPO can not promote the expression of NGB [17].

In our experiment,we observe how clinically application rhEPO effect the expression of NGB in patients with craniocerebral injury. The results showed that the neuroglobin in the rhEPO group is higher than the control group, and with the time changing, the expression of neuroglobin in the rhEPO group of patients increase highly,stating that rhEPO can promote the expression of brain NGB which has the function of neuroprotection. But the mechanisms that how rhEPO induce the expression of NGB is still unclear, remaining to be further confirmed.

Erythropoietin (EPO) is a kind of glycoprotein activation secreted by the kidney, which can improve reticulocyte (RET) counting, red blood cell (RBC), hemoglobin (HB) concentration etc. through stimulating proliferation and differentiation of red bone marrow hematopoietic cells. The study conducted by VanLperen CE [18] showed that the endogenous EPO level of critically ill patients in the ICU in blood is reduced, and the patients can react to exogenous EPO which is reflected by the increasing of reticulocyte (RET) in the blood, so that EPO can be used in the treatment of critically ill patients with anemia.

In this experiment, through clinical application of rhEPO for craniocerebral injury of critically ill patients, we found that after the use of rhEPO, the expression amount of hemoglobin in rhEPO group in serum was obviously higher than that of control group which had explainned that rhEPO can raise the hemoglobin content of critically ill patients in ICU and improve patients with anemia, reduce the rate of blood transfusion.

In this experiment we also monitor the changes of CRP levels in both groups. There have been studies have showed that rhEPO has an adverse effect on the inflammation medium of the body, reducing inflammation of the systemic inflammatory reaction medium like IL 6 and TNF, so as to reduce systemic inflammatory response syndrome [19]; But in our clinical study,we found that the CRP in the two group has no significant difference which may state that rhEPO did not cause the body too high inflammation and is highly safe with small adverse drug reaction. But the relations between rhEPO and CRP remains to be further discussed considering that critically ill patients is complex and inflammation in the body is interfered by various factors.

In this experiment, we observed that the APACHE Ⅱ score in rhEPO group decreased than control group. APACHE Ⅱ scoring system is the most widely clinical applicated and is the most critical condition of authoritative evaluation system in intensive care unit. The scoring system have been researched for many times by many scholars at home and abroad, it can be retrospective evaluation, also can be real-time scored, track dynamic change condition, quantitatively reflect the disease severity, can

objectively assess patients and predict mortality. In this experiment, the rhEPO can make patients APACHE Ⅱ score reduce which may state rhEPO can relieve patients, can improve the prognosis of patients with craniocerebral injury.

The rhEPO can shorten the mechanical ventilation time which can also explain rhEPO can improve the prognosis of patients and have the effect of neuroprotection. Although GCS score of two groups of patients within the treatment time increased, no statistical difference between them.Analyzing the causes, we consider that patients with coma affected by various condition while the response to a single drug rhEPO is small. Looking back the experiment, rhEPO can induce the expression of neuroglobin which has neuroprotection effect, can decrease the APACHE Ⅱ score which can objectively assess the mortality of patients, can shorten the time of patients with mechanical ventilation, and can promote the expression of hemoglobin, provide experimental basis for clinical application of rhEPO, and further confirmed rhEPO neuroprotection effect.

The possible mechanism of EPO to neuroprotection is oxidation resistance--biological activity of rhEPO agree with NO synthetase activity, EPO can inhibit NO synthetase mediated free radicals caused by cell toxicity, thus playing a role of protection of cells. And EPO can increase the activity of antioxidant enzymes, superoxide dismutase (sod), glutathione reductase and catalase to protect neurons. Study confirms that EPO as a nerve protection factor, can play a role of neuroprotection directly and indirectly [20]. Erythropoietin has been proved to have neuro- protection effect against ischemic hypoxic brain injury in cell culture, animal model of brain damage experiment [21]. At the same time, studies have shown that ischemic hypoxic brain damage in patients with early application of erythropoietin can promote angiogenesis and nerve regeneration, with neuroprotection effect [22].

In clinical work, the neuroprotection of patients with craniocerebral injury is still a problem. This study discusses the neuroprotection effect of rhEPO, bring inspiration for clinical work. RhEPO could be the next clinical application effective neural protectant. Foreign scholars have conducted a series researches about the preclinical application of RPO in nervous system diseases, such as stroke and multiple sclerosis, confirmed the validity of the neuroprotection effect of EPO. EPO can not only provide neuroprotection effect in the nervous system emergency and can be used to treat chronic neural function damage and has become the first-line drug candidates in the therapy of nervous system diseases [23]. The clinical effect of EPO is also confirmed by the broad masses of medical researchers step by step. EPO has shifted from the original endocrine hormones that promote erythropoiesis extension to the multi-functional hormone including paracrine and autocrine manner, with the combination of systemic and local effects, can be used as a kind of multi-functional growth factor to maintain the steady state of body internal environment.

Although the research of erythropoietin is more and more deep now, but the clinical application of rhEPO needs further study to solve the problem that unknow. About the question of neuroglobin expression, EPO hormone is just one aspect, but the detailed mechanism of its expression is not entirely clear, still needing further study. For brain injury patients with other diseases, such as tumor, the application of rhRPO still need further confirmed.

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