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Effect of recombinant human erythropoietin therapy on convalescent serological indicators in patients with severe craniocerebral injury

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ABSTRACT

Objective: To study the effect of recombinant human erythropoietin (rHu-EPO) therapy on convalescent serological indicators in patients with severe craniocerebral injury. **Methods:** Patients with severe craniocerebral injury who were treated in Fifth Hospital in Wuhan between July 2014 and February 2017 were selected and randomly divided into the rHu-EPO group who accepted rHu-EPO combined with conventional therapy and the control group who accepted conventional therapy. Before and after treatment, serum levels of nerve injury indexes, inflammation indexes, oxidative stress indexes and apoptosis indexes were measured. **Results:** Serum Tau, S100B, GFAP, NSE, IL-1 β , TNF- α , VCAM-1, ICAM-1, LPO, AOPP, 8-iso-PGF2 , sTRAIL, sFas and sFasL levels of both groups of patients 14 d after treatment were significantly lower than those before treatment, and serum Tau, S100B, GFAP, NSE, IL-1 β , TNF- α , VCAM-1, ICAM-1, LPO, AOPP, 8-iso-PGF2 , sTRAIL, sFas and sFasL levels of rHu-EPO group were significantly lower than those of control group. **Conclusion:** rHu-EPO therapy can significantly improve the convalescent nerve injury, inflammation, oxidative stress and apoptosis in patients with severe craniocerebral injury.

1. Introduction

Craniocerebral injury is a common traumatic disease in neurosurgery, and severe craniocerebral injury is in critical condition, and has high lethality and disability rate[1]. Although emergency decompressive craniectomy and evacuation of hematoma can effectively reduce intracranial pressure and avoid continued hematoma compression on brain tissue, patients will still have different degree of nerve function defect in convalescence[2,3]. In convalescence of patients with severe craniocerebral injury, neurotrophic drugs combined with neural functional exercise can promote the recovery of neural function to a certain extent, but patients still have disturbance of consciousness, body function disorder, language disorders, etc., which seriously affect patients' daily life. Erythropoietin (EPO) was first used in the treatment of anemia, and it has been proven in recent years that it has neuroprotective function, and can promote the growth of neurons and inhibit the inflammatory and oxidative stress reaction in the process of nerve damage[4,5]. In the following study, the effect of recombinant human erythropoietin (rHu-EPO) therapy on convalescent serological indicators in patients with severe craniocerebral injury was analyzed.

2. Patients′ general information and research methods

2.1 General information of severe craniocerebral injury

A total of 48 patients with severe craniocerebral injury who were treated in Fifth Hospital in Wuhan between July 2014 and February 2017 were selected as the research subjects, all patients had a clear history of trauma, and were accompanied by varying degrees of coma and with Glasgow coma scale 6-8 points, and head CT scan confirmed the existence of craniocerebral injury. Patients who were allergic rHu-EPO and had history of cerebrovascular disease were excluded. Random number table was used to divide the 48 patients with severe craniocerebral injury into two groups, 24 cases in each group. The rHu-EPO group included 18 men and 6 women that were 29-52 years old; the control group included 17 men and 7 women that were 32-51 years old. There was no significant difference in general information between the two groups of patients (P>0.05).

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2.2 Clinical treatment methods

Both groups of patients received sedation-analgesia, ice compress on head for physical cooling, mannitol to reduce intracraninal pressure, proton pump inhibitors to protect gastric mucosa, antibiotics to prevent infection and other symptomatic and supportive treatments, as well as decompressive craniectomy, hematoma suction, hematoma removal and other emergency operations if appropriate. Both groups of patients, on the basis of conventional symptomatic treatment, were given intravenous drip of ganglioside and intramuscular injection of mouse nerve growth factor to nourish nerve as well as intravenous drip of edaravone to remove oxygen free radicals, and treatment lasted for 14 consecutive days; rHu-EPO group, based on above treatment, received rHu-EPO therpay, and the method was as follows: they received subcutaneous injection of rHu-EPO 10 000 IU respectively on the 3 d, 6 d, 9 d and 12 d after admission.

2.3 Clinical index detection methods

Before treatment and 14 d after treatment, 8-10 mL of cubital venous blood was collected from two groups of patients and centrifuged at 3 000 r/min to separate upper serum, enzyme-linked immunosorbent assay kit was used to determine serum Tau, S100B, GFAP, NSE, IL-1 β , TNF- α , VCAM-1, ICAM-1, sTRAIL, sFas and sFasL contents, and radioimmunoprecipitation kit was used to determine serum LPO, AOPP and 8-iso-PGF2 levels.

2.4 Statistical methods

SPSS 20.0 software was used to input and analyze data, serum index analysis between two groups was by t test and $P<0.05$ indicated statistical significance in differences.

3. Results

3.1 Serum nerve injury molecule levels

Before treatment and 14 d after treatment, analysis of serum nerve injury molecules Tau (ng/L), S100B (μg/L), GFAP (ng/L) and NSE (μg/L) levels between two groups of patients was as follows: serum Tau, S100B, GFAP and NSE levels were not significantly different between two groups of patients before treatment ($P>0.05$); serum Tau, S100B, GFAP and NSE levels of both groups of patients 14 days after treatment were significantly lower than those before treatment ($P<0.05$), and serum Tau, S100B, GFAP and NSE levels of rHu-EPO group were significantly lower than those of control group ($P<0.05$).

3.2 Serum inflammation molecule levels

Before treatment and 14 d after treatment, analysis of serum inflammation molecules IL-1 β (μg/L), TNF- α (μg/L), VCAM-1 (ng/L) and ICAM-1 (ng/L) levels between two groups of patients was as follows: serum IL-1 β , TNF- α , VCAM-1 and ICAM-1 levels were not significantly different between two groups of patients before treatment ($P>0.05$); serum IL-1 β , TNF- α , VCAM-1 and ICAM-1 levels of both groups of patients 14 days after treatment were significantly lower than those before treatment ($P<0.05$), and serum IL-1 β , TNF- α , VCAM-1 and ICAM-1 levels of rHu-EPO group were significantly lower than those of control group ($P<0.05$).

3.3 Serum oxidative stress molecule levels

Before treatment and 14 d after treatment, analysis of serum oxidative stress molecules LPO (μmol/L), AOPP (μmol/L) and 8-iso-PGF2 (mg/L) levels between two groups of patients was as follows:

Table 1.
Serum nerve injury molecule levels before and after treatment.

Groups	n	Time	Tau	S100B	GFAP	NSE
rHu-EPO group	24	Before treatment	7.39±0.93	0.42±0.07	5.32±0.77	55.29±7.14
		After treatment	4.02±0.56*#	0.18±0.03*#	1.45±0.19*#	25.23±3.58*#
Control group	24	Before treatment	7.51±0.98	0.44±0.08	5.27±0.72	55.78±7.59
		After treatment	5.87±0.77#	0.29±0.05#	2.89±0.35#	37.48±5.14#

*: comparison between rHu-EPO group and control group, $P<0.05$; #: comparison within group before treatment and 14 days after treatment, $P<0.05$.

Table 2.
Serum inflammation molecule levels before and after treatment.

Groups	n	Time	IL-1 β	TNF- α	VCAM-1	ICAM-1
rHu-EPO group	24	Before treatment	2.86±0.36	7.59±0.93	94.52±10.28	146.36±18.56
		After treatment	1.24±0.18*#	3.04±0.55*#	38.59±6.41*#	65.28±8.41*#
Control group	24	Before treatment	2.91±0.39	7.71±0.98	95.12±11.28	148.14±17.59
		After treatment	1.98±0.26#	4.69±0.72#	57.58±8.92#	98.41±11.28#

*: comparison between rHu-EPO group and control group, $P<0.05$; #: comparison within group before treatment and 14 days after treatment, $P<0.05$.

Table 3.
Serum oxidative stress molecule levels before and after treatment.

Groups	n	Time	LPO	AOPP	8-iso-PGF2
rHu-EPO group	24	Before treatment	2.95±0.42	61.92±8.24	0.59±0.08
		After treatment	1.03±0.17*#	26.53±4.45*#	0.15±0.02*#
Control group	24	Before treatment	2.88±0.45	62.31±9.31	0.61±0.09
		After treatment	1.78±0.25#	38.52±5.69#	0.28±0.04#

*: comparison between rHu-EPO group and control group, $P<0.05$; #: comparison within group before treatment and 14 days after treatment, $P<0.05$.

Table 4.
Serum apoptosis molecule levels before and after treatment (μg/L).

Groups	n	Time	sTRAIL	sFas	sFasL
rHu-EPO group	24	Before treatment	109.36±17.46	169.35±19.26	80.25±10.39
		After treatment	47.24±7.14*#	68.15±8.98*#	29.36±4.16*#
Control group	24	Before treatment	110.17±18.15	171.09±17.52	81.67±9.67
		After treatment	78.36±9.98#	42.34±7.25#	42.46±7.42#

*: comparison between rHu-EPO group and control group, $P<0.05$; #: comparison within group before treatment and 14 d after treatment, $P<0.05$.

serum LPO, AOPP and 8-iso-PGF2 levels were not significantly different between two groups of patients before treatment ($P>0.05$); serum LPO, AOPP and 8-iso-PGF2 levels of both groups of patients 14 d after treatment were significantly lower than those before treatment ($P<0.05$), and serum LPO, AOPP and 8-iso-PGF2 levels of rHu-EPO group were significantly lower than those of control group ($P<0.05$).

3.4 Serum apoptosis molecule levels

Before treatment and 14 d after treatment, analysis of serum apoptosis molecules sTRAIL, sFas and sFasL levels between two groups of patients was as follows: serum sTRAIL, sFas and sFasL levels were not significantly different between two groups of patients before treatment ($P>0.05$); serum sTRAIL, sFas and sFasL levels of both groups of patients 14 d after treatment were significantly lower than those before treatment ($P<0.05$), and serum sTRAIL, sFas and sFasL levels of rHu-EPO group were significantly lower than those of control group ($P<0.05$).

4. Discussion

The treatment of patients with severe craniocerebral injury in rehabilitation period is a hot topic in clinical research, and rHu-EPO is a new neuroprotective drug discovered in recent years[6,7]. Animal studies have shown that the expression of EPO significantly reduces in the craniocerebral injury model and is closely related to the extent of the neurological deficit[8]. This suggests that supplementing EPO has therapeutic value for severe craniocerebral injury. In the process of craniocerebral injury, neurons and glial cells will rupture under the sustained damage from mechanical pressure and side metabolites, and then the Tau, S100B, GFAP, NSE and other marker molecules in cells are released into the blood circulation[9-11]. In order to define the effect of rHu-EPO treatment on convalescent neural functional recovery in patients with severe craniocerebral injury, the changes in nerve injury molecule levels were first analyzed in the study, and the results showed that serum Tau, S100B, GFAP and NSE levels of both groups of patients 14 d after treatment were significantly lower than those before treatment, and serum Tau, S100B, GFAP and NSE levels of rHu-EPO group were significantly lower than those of control group. This shows that the rHu-EPO treatment is more effective than conventional treatment in reducing the degree of nerve damage in patients with severe craniocerebral injury.

In the course of craniocerebral injury, inflammation is an important pathological link that causes neural functional secondary damage, and the persistence of damaged local hematoma tissue will significantly activate inflammatory reaction and cause the secretion of a variety of inflammatory mediators, which cause neural functional secondary damage on the basis of neurological damage directly caused by mechanical factors[12,13]. IL-1β, TNF-α, VCAM-1 and ICAM-1 are the inflammatory mediators that are closely associated with local inflammatory response after craniocerebral injury. IL-1β is the most abundant in brain cell tissue space and in cerebrospinal fluid, which is an important mediator mediating inflammatory response and secondary injury in the brain tissue; TNF-α is the pro-inflammatory factor that first changes during the inflammatory response, and it can recruit multiple inflammatory cells in local arae and amplify the inflammatory response; VCAM-1 and ICAM-1 are the important adhesion molecules that can mediate the adhesion and infiltration of inflammatory cells within the lesion, thereby promoting cascade activation of inflammatory response. EPO can inhibit the activation of the inflammatory response and the secretion of inflammatory factors, and analysis of the changes in above inflammatory response molecule levels in serum in the study showed that serum IL-1β, TNF-α, VCAM-1 and ICAM-1 levels of both groups of patients 14 d after treatment were significantly lower than those before treatment, and serum IL-1β, TNF-α, VCAM-1 and ICAM-1 levels of rHu-EPO group were significantly lower than those of control group. This means that the rHu-EPO treatment is more effective than conventional treatment in reducing the inflammatory response in patients with severe craniocerebral injury.

EPO not only has anti-inflammatory activity, but also has the biological effect of anti-oxidative stress. Oxidative stress is an

important secondary pathological factor causing craniocerebral injury, local hematoma compression can lead to increased release of oxygen free radicals, and oxygen free radicals have oxidizing reaction with the multiple ingredients on the cell membrane and biofilm, and cause damage to cells and organelles[14]. The lipid in the cell membrane and biofilm reacts with oxygen free radicals and generates LPO, and lipodized arachidonate reacts with oxygen free radicals and generates 8-iso-PGF2 ; the protein in the cells reacts with oxygen free radicals and produces AOPP[15,16]. In order to define the rHu-EPO effect on convalescent oxidative stress reaction in patients with severe craniocerebral injury, serum levels of above oxidative stress products were analyzed in the study, and the results showed that serum LPO, AOPP and 8-iso-PGF2 levels of both groups of patients 14 d after treatment were significantly lower than those before treatment, and serum LPO, AOPP and 8-iso-PGF2 levels of rHu-EPO group were significantly lower than those of control group. This suggests that rHu-EPO therapy is more effective than conventional treatment in reducing the degree of oxidative stress response in patients with severe craniocerebral injury.

The activation of local inflammation and oxidative stress reaction in patients with craniocerebral injury can not only directly cause damage in neurons and glial cells, but can also start the apoptosis and cause cellular damage. Fas/FasL is able to trigger the caspase-8/caspase-3 cascade activation through the combination of the receptor-ligand, causing cell apoptosis[17]; TRAIL is a new type of inducer of apoptosis, and sTRAIL is its soluble form that can cause cell apoptosis when it is delivered to the lesion with the blood circulation[18]. In order to define the rHu-EPO effect on nerve cell apoptosis when it was used for convalescent treatment of patients with severe craniocerebral injury, the changes in serum levels of above apoptosis molecules were analyzed in the study, and the results showed that serum sTRAIL, sFas and sFasL levels of both groups of patients 14 d after treatment were significantly lower than those before treatment, and serum sTRAIL, sFas and sFasL levels of rHu-EPO group were significantly lower than those of control group. This suggests that rHu-EPO therapy may be more effective than conventional treatment in suppressing the nerve cell apoptosis in patients with severe craniocerebral injury.

To sum up, it is believed that rHu-EPO treatment could alleviate convalescent nerve injury, inflammation, oxidative stress and cell apoptosis in patients with severe craniocerebral injury, and it has positive value for disease recovery.

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