Int J Clin Exp Med 2016;9(11):22267-22274
www.ijcem.com /ISSN:1940-5901/IJCEM0028191

Original Article

The effect of erythropoietin in the treatment of acute spinal cord injury

Zhaohui Cheng[1,2*], Tiao Lin[3*], Weishan Chen[1], Weigang Wu[1], Shigui Yan[1]

[1]Department of Orthopaedic Surgery, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, Zhejiang, China; [2]Department of Orthopaedic Surgery, Huangyan Hospital, Wenzhou Medical College, Taizhou 318020, Zhejiang, China; [3]Musculoskeletal Oncology Center, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510080, Guangdong, China. [*]Equal contributors.

Received March 14, 2016; Accepted September 16, 2016; Epub November 15, 2016; Published November 30, 2016

Abstract: Objectives: The aim of this study was to evaluate the recovery of motor and sensory function in patients with acute spinal cord injury (ASCI) after recombinant human erythropoietin (EPO) treatment. The safety and efficacy of EPO for ASCI was discussed as well. Methods: The clinical data of 60 ASCI cases were analyzed in a retrospective manner, in which EPO-treated therapy group (n=30) and the EPO-untreated group (n=30) were included. The neurological function of each patient at admission, twelve and twenty-fourth months were reviewed. The adverse events were recorded as well. Results: All of the 60 patients were followed up for 2~3 years (average 2.4 years) after treatment. The follow-up of both groups showed that the patients in the therapy group had a better improvement in the AIS and ASIA motor score and more sensation of touch than the control group ($P<0.05$). No statistically significant difference regarding the red blood cell count and hemoglobin (Hb) concentration between therapy group and control group was found ($P>0.05$). Conclusion: EPO is a safe and effective drug for treating ASCI. Early application of EPO can promote the recovery of the motor and sensory function in patients with ASCI.

Keywords: Acute spinal cord injury, erythropoietin, neurological function

Introduction

Acute spinal cord injury (ASCI) is a serious clinical condition with high morbidity and mortality. It is reported that there were more than 11,000 new cases of SCI each year in USA, over half of which occur among individuals under 30 years of age [1-3]. Due to its unknown pathogenesis, ASCI is related with extremely high costs and poor clinical outcomes, and continues to be a large persistent burden to patients and society [1-5].

Although the pathogenesis of ASCI is not fully understood, there is growing evidence showing that ASCI is a complex process involving oxidative stress, and inflammatory response, which leads to destruction of neuronal tissue and vascular structure [6, 7]. The main strategy to promote recovery following ASCI is to reduce the secondary injury that was induced by the activated and released toxic substances including lipid peroxidase, glutamate, vasoactive eicosanoids, and free radicals [8-11].

Methylprednisolone sodium succinate (MPSS) is currently the most widely used drug for ASCI, and is still used as a standard treatment for ASCI in many countries to limit secondary effects of trauma [12]. However, MPSS is related with high incidence of side effects on the respiratory system and digestive systems. Its clinical efficacy, also remains a great controversy [13]. Hence, new pharmacological agents to replace MPSS are needed. Recently, an increasing number of therapies for ASCI have been emerging from the laboratory and are pursuing translation into human clinical trials.

A growing number of studies have demonstrated that erythropoietin (EPO), a secreted 30-kD glycoprotein, provides substantial benefits to ASCI [14-18]. EPO and its receptors are ubiquitously expressed in the central and peripheral nervous system, where it regulates the development of the central nervous system and exerts neurotrophic and neuroprotective effects, as well as anti-apoptotic, anti-oxidantand anti-inflammatory effects through multiple signaling

Table 1. General information of patients in two groups before treatment

Groups	Cases	Gender	Age (± SD)	AIS			
		(Male/female)	(Years old)	A	B	C	D
Therapy	30	18/12	39.3 ± 11.2	15	5	7	3
Control	30	16/14	38.5 ± 13.1	16	7	5	2
P		0.60	0.80	0.83			

pathways including activating NF-κB pathway, MAP kinase pathway, and STAT5 pathway. In animal studies, the administration of recombinant human EPO (rhEPO) in a rat model of ASCI significantly improves functional outcome [19-21]. However, few study has been reported about the clinical efficacy of EPO in the treatment of ASCI [22]. We performed this retrospective cohort study to evaluate the safety and efficacy of EPO by comparing the neurological function improvement between the therapy group and control group.

Materials and methods

General information

This retrospective study was approved by the Ethics Committee of The Second Affiliated Hospital, School of Medicine, Zhejiang University. The definition of ASCI was based on previously reported studies [22-24]. Patients who were: (1) those who had ASCI, (2) being admitted into our department 10~60 hours after the trauma, (3) blood hemoglobin (Hb) ≤15.0 g/dL were included in this study. Patients who were: (1) those with involvement of cauda equina of nerve root only, multiple trauma, penetrating wounds, (2) those who received steroids, treatment with erythropoietin in the past 30 days, (3) receiving immunosuppressive drugs, (3) and age under 18 years were excluded in this study.

A total of 60 patients with various symptoms of traumatic ASCI admitted into our department from December 2009 to December 2011 were reviewed, of which thirty patients were treated with EPO and the rest thirty were EPO-untreated. The characteristics of the included patients in both groups were showed in **Table 1**. Injuries were graded according to the ASIA Impairment Scale (AIS). The gender ratio, age and initial AIS in the two groups were comparable, and the differences between them were unobvious (P>0.05), (**Table 1**).

Therapeutic method

All patients' vital signs were monitored and were given symptomatic relief and supportive treatment. Patients admission time varied from 10~60 h (average 26 h).

Similar surgical procedures were performed by the same surgical team within three days of admission in all 60 patients, each group included: 7 cases of cervical corpectomy and titanium mesh bone-graft fusion internal fixation; 11 cases of anterior cervical diskectomy, fusion cage and plate fixation; 2 cases of combined anterior and posterior approaches (posterior restoration, lateral mass screw fixation+ anterior cervical diskectomy, fusion cage and plate fixation); 10 cases with thoracolumbar vertebrae decompression laminectomy and pedicle screw-rod fixation. All the surgical procedures were performed under general anesthesia and there was no difference of the anesthesia technique for all patients. Similar post-surgery treatments including hemostatic, acid-suppressive, dehydration, and anti-inflammation therapies (cefuroxime at a dose of 1.5 g twice a day for two days) were performed in all patients. For patients with gastrointestinal problems, symptomatic treatment including acid-suppressive and anti-constipation medications were used. For patients with urinary problems like urinary infection, sensible antibiotics based on bacterial cultivation were used. For patients with hypotension, volume resuscitation with crystalloid was applied. There was no difference in the common post-surgery treatment between groups. MPSS was not applied in any of included patients. The rhEPO was provided in 4000 unit vials (Yi Biao; Shenyang Sansheng Pharmaceutical Co., Ltd.). Based on our previous studies [25-29] and the drug use instructions, the therapy group received additional daily intravenous infusion (intravenously guttae) of rhEPO at 12,000 IU/day (in 100 ml normal saline during 30 minutes), for 10 days, which is also consistent with previous reported models of induced SCI in animals and the previous systematic review of controlled trials in animal models about the application of EPO on nervous system injury [30]. The patients in control group received the same infusion of saline without EPO. The rehabilitation and formal physical therapy started after 24 hours of surgery. After the treatment in the department of orthopedic surgery was finished, patients

Table 2. AIS in two groups before and after treatment

Items	Therapy group (cases)					Control group (cases)				
	A	B	C	D	E	A	B	C	D	E
Admission	15	5	7	3	0	16	7	5	2	0
Follow-up (1 Y)	3	6	7	7	7	5	8	8	4	5
Follow-up (2 Y)	2	6	7	8	8	4	8	9	4	5

were transferred to the department of rehabilitation and underwent the readaptation program including range of motion training, gait training, muscle strengthening, and activities of daily living training [31]. The postoperative review of the X-ray and CT scan confirmed stable internal fixation and good vertebral sequence. All of the 60 patients were followed up for 2 to 3 years, with an average of 2.4 years.

Neurological function scoring criteria

The efficacy of the treatment was predicated on performing an accurate inspection, according to the ASIA 2000 scoring criteria [32], at admission, 1-year and 2-year follow-up examinations. AIS: A=Complete: No sensory or motor function is preserved in the S4-S5 sacral segments. B=Incomplete: Sensory function is preserved but motor function is impaired below the neurological level and includes the S4-S5 sacral segments. C=Incomplete: Motor function is preserved, while more than half of key muscles are impaired below the neurological level, with a muscle grade less than 3. D=Incomplete: Motor function is preserved, while more than half of key muscles below the neurological level have a muscle grade of 3 or more. E=Normal: motor and sensory functions are normal.

The motor function was evaluated by examining the function of a key muscle within each of 10 myotomes on both sides of body and by assessing the muscle strength using a 0~5 clinical classification scale, the maximum score of each extremity is 25, totaling 100 for upper and lower limbs. The sensory examination was performed, from C_2~S_5, by examining key sensory function within each of 28 dermatomes on both sides of the body and by scoring them according to three grades (0=absent; 1=altered; 2=normal); the maximum score of pin prick modalities and light touch is 56 points, totaling 112 points per side of the body.

Blood routine examination and monitoring serum EPO levels

The blood routine examination and the liver (aspartate transaminase, alanine transaminase) and kidney function (Cr, blood urine nitrate) tests were performed on admission and treatment and 14 days after that. Enzyme-linked immunosorbent assay (ELISA) was used to measure the EPO level in serum. Close attention was paid to the complications of the cardiovascular system, lung infections, urinary tract infections, and stress ulcers.

Statistical methods

The Statistical Product and Service Solutions (SPSS) 18.0 statistical software was used for the analysis of the results. The data were expressed as mean ± standard deviation ($\bar{x}$ ± s). Comparison between the two groups was done with the independent sample T-test and correlation analysis. $P<0.05$ was considered significantly different.

Results

Comparison of AIS in two groups before and after treatment

The two groups were divided into four grades, according to different function series before and after treatment. For no change before and after treatment the curative effect was considered as grade 0; a one-level curative effect of treatment progress, compared with before treatment, was considered as grade 1; a two-level curative effect of progress was considered as grade 2; and a three-level curative effect of progress was considered as grade 3. The two groups of AIS grades were compared and analyzed by x^2 test. No significant difference was detected of the two groups of initial AIS ($P>0.05$) (**Table 2**). At the 1-year follow-up, when the AIS grade increased was 1 (or more) grade, the EPO-treated group was 90% (27/30) and the control group was 83% (25/30), and the difference between them was not significant ($P>0.05$); when the AIS recovery grade was 2 (or more), the EPO-treated group was 47% (14/30) and the control group was 20% (6/30), and the difference was statistically significant ($P<0.05$); when the AIS grade increased was 3 grade, the EPO-treated group was 6.7% (2/30) and the control group was 0% (0/30), and the difference between them was

Table 3. The grade comparison of nerve functional restoration (Follow-up)

Group	Cases	Unrecovered	Grade one	Grade two	Grade three
Therapy (1 Y)	30	3	13	12	2
Therapy (2 Y)	30	2	12	13	3
Control (1 Y)	30	5	19	6	0
Control (2 Y)	30	4	18	7	1

not significant (P>0.05); At the 2-year follow-up, when the AIS grade recovery was 2 (or more), the EPO-treated group was 53.3% (16/30) and the control group was 26.7% (8/30), and the difference was statistically significant (P<0.05); when the AIS recovery grade was 3, the EPO-treated group was 10% (3/30), and the control group was 3.3% (1/30), and the difference between them was not significant (P>0.05) (**Table 3**).

Comparison of the ASIA scores of motor, pin prick and light touch in the two groups before and after treatment

The comparison of the ASIA scores data revealed that the differences in the Motor, Pin Prick and Light Touch between the two groups, on admission, were not statistically significant (P>0.05). At the 1-year and 2-year follow-up, the neurological status in the two groups of patients was determined to be stable, and the Motor, Pin Prick and Light Touch scores were improved in varying degrees. However, the neurological recovery of the patients in the treatment group was better than those in the control group, and the improvement in motor function for patients in the treatment group was obvious, as indicated by the differences between the groups which were found to be statistically significant (P<0.05) (**Table 4**).

Comparison of the routine blood examination results, EPO levels in the two patient groups

The results of the blood routine examination (complete blood count), the serum EPO levels, and the liver (aspartate transaminase, alanine transaminase) and kidney function (Cr, blood urine nitrate) tests in all patients, on admission, were all in the normal range. However, a small number of patients had reduced hemoglobin and elevated transaminases, which was considered as a stress response. The serum EPO level in the therapy group was significantly increased on the seventh day (ΔP<0.01), whereas it was not significantly changed in the control group. The red blood cell count and hemoglobin concentration in the therapy group were slightly higher than in the control group, but the differences were not statistically significant (P>0.05) (**Table 5**). No significant side effect was noted during the study period.

Discussion

ASCI is usually caused by external trauma, followed by dislocation of the spine and rupture of the intervertebral disc. Although many therapies are administered as soon as possible after ASCI with the hope of attenuating secondary damage and maximizing the sparing of neurological tissue, the therapeutic effect is unsatisfactory, and ASCI is still related with extremely high costs and poor clinical outcomes. In this study, we explored the feasibility, safety and efficacy of EPO for ASCI treatment by retrospectively analyzing clinical data of 60 cases. To the best of our knowledge, this is the first clinical study about the use of EPO (>6 hour from the injury) on ASCI. Through evaluating the recovery of motor and sensory function in patients with ASCI after treatment with rhEPO, we found that the rhEPO treatment significantly improved the AIS and ASIA motor score and the sensation of touch when compared with the control group (P<0.05), while no statistically significant change regarding the red blood cell count and hemoglobin (Hb) concentration was found (P>0.05). The results obtained here indicate that early application of EPO may improve neurological function in patients with ASCI.

Motor and sensory function are two of the most important parameters of function recovery of patients with ASCI. The results of functional scores in this study are in accordance with previous reported animal studies in experimental models of SCI [7, 33-36]. Cerri and colleagues [33] found that compared with saline-treated controls, EPO-treated animals experienced a better general improvement both in sensory and motor transmission through spared spinal pathways, supposedly via the reticulo-spinal system. The Non-behavioral outcomes included improved sparing of white and grey matter, reduced apoptosis and lipid peroxidation, reduced ERK phosphorylation, and decreased inflammatory cytokine release and

Table 4. Changes in the ASIA scores of the Motor, Light Touch and Pin Prick in the two groups before and after treatment ($\bar{x} \pm s$)

Group	Cases	Admission			Follow-up (1 Y)			Follow-up (2 Y)		
		Motor	Light Touch	Pin Prick	Motor	Light Touch	Pin Prick	Motor	Light Touch	Pin Prick
Therapy	15	38.3 ± 7.6	54.2 ± 7.5	55.4 ± 6.6	55.2 ± 8.0	74.7 ± 9.6	79.6 ± 12.1	55.3 ± 7.7	75.5 ± 11.1	79.5 ± 10.6
Control	15	37.5 ± 8.2	56.8 ± 8.8	58.2 ± 7.2	46.6 ± 4.8	65.4 ± 11.3	68.7 ± 8.9	47.7 ± 5.8	65.5 ± 12.3	70.7 ± 11.4
t		0.37	1.21	1.55	5.03	3.42	3.77	4.30	3.29	3.09
P		>0.05	>0.05	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 5. Comparison of blood routine examination, EPO level in the two groups

Groups	Cases	On admission			After treatment for 14 Days		
		RBC (10^{12}/L)	Hb (g/L)	EPO (mu/ml)	RBC (10^{12}/L)	Hb (g/L)	EPO (mu/ml)
Therapy	30	4.33 ± 0.55	112 ± 25.8	8.55 ± 5.3	4.90 ± 0.45	121 ± 30.5	204.6 ± 42.8$^{\Delta}$
Control	30	4.53 ± 0.53	118 ± 22.5	7.82 ± 5.6	4.66 ± 0.58	115 ± 25.7	8.67 ± 4.6
t		1.41	0.94	0.50	1.77	0.80	24.91
P		>0.05	>0.05	>0.05	>0.05	>0.05	<0.001

$^{\Delta}$p<0.01.

neutrophil invasion [37]. Equally relevant, Freitag MT and colleagues [34] found that the therapeutic effect of EPO in early SCI that leads to a significant recovery in rats, a significantly reduced immune response and a significantly reduced number of apoptotic cells at the height of the lesion epicenter. Furthermore, a dose effect was demonstrated in experiments by both Gorio and colleagues [7] and Kontogeorgakos and colleagues [35] in which different doses of EPO were tested intravenously or subcutaneously separately, the optimal results were observed with the lower doses. Incidentally, Rangarajan V and colleagues [36] believe that the scientific rationale and preclinical data for erythropoietin neuroprotection are promising.

The results of our study are also in agreement with the previous reported clinical trial [22]. Alibai et al. conducted a randomized controlled double-blind clinical trial and evaluated the effect of rhEPO plus MPSS compared to MPSS alone to improve neurological function of patients after ASCI. In their study, MPSS plus rhEPO started within 6 hours after ASCI significantly improved of neurologic function in one week (P=0.046), one month (P=0.021) and six months (P=0.018) after admission when compared with MPSS plus placebo treated patients [22]. It has been reported that one dose EPO was enough to improve neurological outcome if being used shortly after the injury but multiple doses were more effective when the treatment was delayed [38]. In our study, we included patients 10~60 h (average 26 h) from the injury, together with our previous studies [25-29], we used ten doses of rhEPO with 24 hours interval. The 12000 IU maintenance dose used in this study is relatively high but within the safe tolerance range. However, rhEPO as an effective neuroprotective drugs has a relatively high potential for translation due to the fact that it is already used in human clinical applications [37]. The high serum EPO level in patients of the control group is due to a natural negative feedback mechanism response activated by the body's loss of blood. However, due to the low endogenous production of EPO after spinal cord injury, the EPO levels are insufficient to trigger the appropriate cell signaling transduction pathways and generate the response leading to inhibition of nerve cell apoptosis. Accordingly, the sensory and motor functions of the patients in the control group were significantly reduced compared with the EPO-treated group patients. Therefore, we recommend that, after ASCI, EPO should be used as soon as possible and maintained for 10~14 d, which together with positive early surgical decompression should provide good conditions for the neurological recovery of the patient.

Besides the promising tissue protective effects, EPO has also adverse effects including polycythemia, hypertension accident, allergic reactions, liver damage and gastrointestinal

discomfort. The results of this study showed that after the application of EPO, the serum EPO concentration increased significantly in the treated patients, whereas the change in the control group is not noticeable. However, by monitoring the patients' blood pressure, blood routine tests and serum EPO concentration before and after treatment, we didn't encounter any clinically significant adverse effects such as thromboembolic phenomenon. The hemoglobin concentration and hematocrit were within the normal range in both groups. More and larger clinical trials in the future would be helpful to confirm the efficacy and safety of EPO in ASCI.

Conclusions

Based on our study, early application of EPO may improve neural function in patients with ASCI, promote the recovery of useful functions, speed up the patient's rehabilitation, and improve the quality of life. However, the study is limited because of group size and not being prospective. Future studies about the aging-concentration-response relationship of EPO for spinal cord protection, and the individual differences in drug use will contribute to the further application of EPO for spinal cord injury.

Acknowledgements

At the point of finishing this paper, I'd like to express my sincere thanks to all the patients who were involved in this study. This project was supported by Huangyan Science and Technology Bureau (Science and Technology Project 2010060 to Cheng).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Shigui Yan, Department of Orthopaedic Surgery, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, China. E-mail: zrjwsj@zju.edu.cn

References

[1] Wong BR, Rho J, Arron J, Robinson E, Orlinick J, Chao M, Kalachikov S, Cayani E, Bartlett FS 3rd, Frankel WN, Lee SY and Choi Y. TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. J Biol Chem 1997; 272: 25190-25194.

[2] Savitsky E and Votey S. Emergency department approach to acute thoracolumbar spine injury. J Emerg Med 1997; 15: 49-60.

[3] Schwab ME. Repairing the injured spinal cord. Science 2002; 295: 1029-1031.

[4] Pimentel L and Diegelmann L. Evaluation and management of acute cervical spine trauma. Emerg Med Clin North Am 2010; 28: 719-738.

[5] Pickelsimer E, Shiroma EJ and Wilson DA. Statewide investigation of medically attended adverse health conditions of persons with spinal cord injury. J Spinal Cord Med 2010; 33: 221-231.

[6] Gelain F, Panseri S, Antonini S, Cunha C, Donega M, Lowery J, Taraballi F, Cerri G, Montagna M, Baldissera F and Vescovi A. Transplantation of nanostructured composite scaffolds results in the regeneration of chronically injured spinal cords. ACS Nano 2011; 5: 227-236.

[7] Gorio A, Gokmen N, Erbayraktar S, Yilmaz O, Madaschi L, Cichetti C, Di Giulio AM, Vardar E, Cerami A and Brines M. Recombinant human erythropoietin counteracts secondary injury and markedly enhances neurological recovery from experimental spinal cord trauma. Proc Natl Acad Sci U S A 2002; 99: 9450-9455.

[8] Pannu R, Barbosa E, Singh AK and Singh I. Attenuation of acute inflammatory response by atorvastatin after spinal cord injury in rats. J Neurosci Res 2005; 79: 340-350.

[9] Bracken MB, Shepard MJ, Holford TR, Leo-Summers L, Aldrich EF, Fazl M, Fehlings M, Herr DL, Hitchon PW, Marshall LF, Nockels RP, Pascale V, Perot PL Jr, Piepmeier J, Sonntag VK, Wagner F, Wilberger JE, Winn HR and Young W. Administration of methylprednisolone for 24 or 48 hours or tirilazad mesylate for 48 hours in the treatment of acute spinal cord injury. Results of the Third National Acute Spinal Cord Injury Randomized Controlled Trial. National Acute Spinal Cord Injury Study. JAMA 1997; 277: 1597-1604.

[10] Pointillart V, Petitjean ME, Wiart L, Vital JM, Lassie P, Thicoipe M and Dabadie P. Pharmacological therapy of spinal cord injury during the acute phase. Spinal Cord 2000; 38: 71-76.

[11] Pollard ME and Apple DF. Factors associated with improved neurologic outcomes in patients with incomplete tetraplegia. Spine (Phila Pa 1976) 2003; 28: 33-39.

[12] Hawryluk GW, Rowland J, Kwon BK and Fehlings MG. Protection and repair of the injured spinal cord: a review of completed, ongoing, and planned clinical trials for acute spinal cord injury. Neurosurg Focus 2008; 25: E14.

[13] Hugenholtz H. Methylprednisolone for acute spinal cord injury: not a standard of care. CMAJ 2003; 168: 1145-1146.
[14] Kwon BK, Fisher CG, Dvorak MF and Tetzlaff W. Strategies to promote neural repair and regeneration after spinal cord injury. Spine (Phila Pa 1976) 2005; 30: S3-13.
[15] Grasso G, Sfacteria A, Meli F, Passalacqua M, Fodale V, Buemi M, Giambartino F, Iacopino DG and Tomasello F. The role of erythropoietin in neuroprotection: therapeutic perspectives. Drug News Perspect 2007; 20: 315-320.
[16] King VR, Averill SA, Hewazy D, Priestley JV, Torup L and Michael-Titus AT. Erythropoietin and carbamylated erythropoietin are neuroprotective following spinal cord hemisection in the rat. Eur J Neurosci 2007; 26: 90-100.
[17] Jin W, Ming X, Hou X, Zhu T, Yuan B, Wang J, Ni H, Jiang J, Wang H and Liang W. Protective effects of erythropoietin in traumatic spinal cord injury by inducing the Nrf2 signaling pathway activation. J Trauma Acute Care Surg 2014; 76: 1228-1234.
[18] Nichol A, French C, Little L, Haddad S, Presneill J, Arabi Y, Bailey M, Cooper DJ, Duranteau J, Huet O, Mak A, McArthur C, Pettila V, Skrifvars M, Vallance S, Varma D, Wills J and Bellomo R. Erythropoietin in traumatic brain injury (EPO-TBI): a double-blind randomised controlled trial. Lancet 2015; 386: 2499-2506.
[19] Siren AL, Fratelli M, Brines M, Goemans C, Casagrande S, Lewczuk P, Keenan S, Gleiter C, Pasquali C, Capobianco A, Mennini T, Heumann R, Cerami A, Ehrenreich H and Ghezzi P. Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress. Proc Natl Acad Sci U S A 2001; 98: 4044-4049.
[20] Digicaylioglu M and Lipton SA. Erythropoietin-mediated neuroprotection involves cross-talk between Jak2 and NF-kappaB signalling cascades. Nature 2001; 412: 641-647.
[21] Zhu L, Wang HD, Yu XG, Jin W, Qiao L, Lu TJ, Hu ZL and Zhou J. Erythropoietin prevents zinc accumulation and neuronal death after traumatic brain injury in rat hippocampus: in vitro and in vivo studies. Brain Res 2009; 1289: 96-105.
[22] Alibai E, Zand F, Rahimi A and Rezaianzadeh A. Erythropoietin plus methylprednisolone or methylprednisolone in the treatment of acute spinal cord injury: a preliminary report. Acta Med Iran 2014; 52: 275-279.
[23] Fehlings MG, Nakashima H, Nagoshi N, Chow DS, Grossman RG and Kopjar B. Rationale, design and critical end points for the Riluzole in Acute Spinal Cord Injury Study (RISCIS): a randomized, double-blinded, placebo-controlled parallel multi-center trial. Spinal Cord 2016; 54: 8-15.
[24] Hu Y, Zheng QX, Guo XD, LJ, Liu Y, Liu GJ, Lian K. The treatment of spinal cord injury with recombinant human erythropoietin. Chin J Exp Surg 2009; 1: 26.
[25] Zheng QX, WY, et al. Protective effect of recombinant-Human Erythropoietin on neuronal apoptosis after spinal cord injury in rats. Chin J Rehabil Theory Practice 2005; 11.
[26] Wang JG, ZQ, Guo XD. An experimental study of rHu-EPO and sodium b-Aescin on the treatment of spinal cord injury in rats. Chinese Journal of Spine Cord 2003; 13: 674-677.
[27] Guo XQ, ZQ. The modulation of erythropoietin and methylprednisolone on the adrenomedullin expression in acute spinal cord injured tissue of rats. Chinese Journal of Clinical Rehabilitation Medicine 2007; 22: 503-505.
[28] Wang JG ZQ, Wang YT, Zhao M. Effect of recombinant human erythropoietin on neural cell apoptosis and related cytokine expression in rats with spinal cord injury. Chinese Journal of Clinical Rehabilitation Medicine 2005; 9.
[29] Guo Xinqing ZQ. Expression of adrenomedullin in acute spinal cord injury tissue of rats and the interventional effects of erythropoitin. Chinese Journal of Clinical Rehabilitation Medicine 2006; 10.
[30] Peng W, Xing Z, Yang J, Wang Y, Wang W and Huang W. The efficacy of erythropoietin in treating experimental traumatic brain injury: a systematic review of controlled trials in animal models. J Neurosurg 2014; 121: 653-664.
[31] Marca L, Sipski SR. Spinal cord injury rehabilitation: State of the science. Am J Phys Med Rehabil 2006; 85: 310-342.
[32] Hayes KC, Hsieh JT, Wolfe DL, Potter PJ and Delaney GA. Classifying incomplete spinal cord injury syndromes: algorithms based on the International Standards for Neurological and Functional Classification of Spinal Cord Injury Patients. Arch Phys Med Rehabil 2000; 81: 644-652.
[33] Cerri G, Montagna M, Madaschi L, Merli D, Borroni P, Baldissera F and Gorio A. Erythropoietin effect on sensorimotor recovery after contusive spinal cord injury: an electrophysiological study in rats. Neuroscience 2012; 219: 290-301.
[34] Freitag MT, Marton G, Pajer K, Hartmann J, Walder N, Rossmann M, Parzer P, Redl H, Nogradi A and Stieltjes B. Monitoring of Short-Term Erythropoietin Therapy in Rats with Acute Spinal Cord Injury Using Manganese-Enhanced Magnetic Resonance Imaging. J Neuroimaging 2015; 25: 582-589.
[35] Kontogeorgakos VA, Voulgaris S, Korompilias AV, Vekris M, Polyzoidis KS, Bourantas K and Beris AE. The efficacy of erythropoietin on acute spinal cord injury. An experimental study

on a rat model. Arch Orthop Trauma Surg 2009; 129: 189-194.

[36] Rangarajan V and Juul SE. Erythropoietin: emerging role of erythropoietin in neonatal neuroprotection. Pediatr Neurol 2014; 51: 481-488.

[37] Kwon BK, Okon E, Hillyer J, Mann C, Baptiste D, Weaver LC, Fehlings MG and Tetzlaff W. A systematic review of non-invasive pharmacologic neuroprotective treatments for acute spinal cord injury. J Neurotrauma 2011; 28: 1545-1588.

[38] Brines M CA. Erythropoietin in spinal cord injury. Novel Therapeutic Options for Neuroprotection 2006; 147-164.