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

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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-oxidant and anti-inflammatory effects through multiple signaling pathways.
pathways including activating NF-kB 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 disectomy, fusion cage and plate fixation; 2 cases of combined anterior and posterior approaches (posterior restoration, lateral mass screw fixation+ anterior cervical disectomy, fusion cage and plate fixation); 10 cases with thoracolumbar vertebra 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>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>Gender (Male/female)</th>
<th>Age (± SD)</th>
<th>AIS A</th>
<th>AIS B</th>
<th>AIS C</th>
<th>AIS D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therapy</td>
<td>30</td>
<td>18/12</td>
<td>39.3 ± 11.2</td>
<td>15</td>
<td>5</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>16/14</td>
<td>38.5 ± 13.1</td>
<td>16</td>
<td>7</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>P</td>
<td>0.60</td>
<td>0.80</td>
<td>0.83</td>
<td></td>
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</tbody>
</table>

**Table 1.** General information of patients in two groups before treatment
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 (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 x² 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 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 2. AIS in two groups before and after treatment

<table>
<thead>
<tr>
<th>Items</th>
<th>Therapy group (cases)</th>
<th>Control group (cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A  B  C  D  E</td>
<td>A  B  C  D  E</td>
</tr>
<tr>
<td>Admission</td>
<td>15  5  7  3  0</td>
<td>16  7  5  2  0</td>
</tr>
<tr>
<td>Follow-up (1 Y)</td>
<td>3   6  7  7  7</td>
<td>5   8  8  4  5</td>
</tr>
<tr>
<td>Follow-up (2 Y)</td>
<td>2   6  7  8  8</td>
<td>4   8  9  4  5</td>
</tr>
</tbody>
</table>

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 C₂~S₅, 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.

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.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Admission</th>
<th>Follow-up (1 Y)</th>
<th>Follow-up (2 Y)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Motor</td>
<td>Motor</td>
<td>Motor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Light Touch</td>
<td>Light Touch</td>
<td>Light Touch</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pin Prick</td>
<td>Pin Prick</td>
<td>Pin Prick</td>
</tr>
<tr>
<td>Therapy</td>
<td>15</td>
<td>38.3 ± 7.6</td>
<td>54.2 ± 7.5</td>
<td>55.4 ± 6.6</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>37.5 ± 8.2</td>
<td>56.8 ± 8.8</td>
<td>58.2 ± 7.2</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
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<tr>
<td>P</td>
<td></td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>On admission</th>
<th>After treatment for 14 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RBC (10(^12)/L)</td>
<td>Hb (g/L)</td>
</tr>
<tr>
<td>Therapy</td>
<td>30</td>
<td>4.33 ± 0.55</td>
<td>112 ± 25.8</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>4.53 ± 0.53</td>
<td>118 ± 22.5</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>1.41</td>
<td>0.94</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

\(p<0.01\)

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.

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Disclosure of conflict of interest

None.

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References

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